A METHOD FOR THE SIMULTANEOUS DETERMINATION OF BLOOD GROUP AND PLATELET ANTIGEN GENOTYPES

TECHNICAL FIELD

This invention relates to an ultra high throughput (UHT) multiplex PCR genotyping method. More specifically, the present invention relates to an automated method of determining a plurality of blood group and platelet antigen, preferably human platelet antigen (HPA), genotypes simultaneously from a single sample through the detection of single nucleotide polymorphisms (SNPs) for various blood group and platelet antigens.

BACKGROUND OF THE INVENTION

At present, there are 29 blood group systems and 6 HPA systems recognized by the International Society of Blood Transfusion (ISBT), wherein, with a few exceptions, a blood group 'system' may be defined by a single gene at a given locus of the human genome (Daniels, G.L. et al. Vox Sang 2003;84:244; Metcalfe P. et al., Vox Sang. 2003;85:240). Most people know their ABO and Rh blood group. However, the ABO and Rh blood group systems expressed on red cells simply represent antigens from only two of the 29 blood group systems, and more systems are being discovered each year. Some examples of blood group systems are the ABO, Rh (D, C, c, E, e), P, Lutheran, Kell (K, k), Lewis, Duffy (Fy^a, Fy^b), or Kidd (Jk^a, Jk^b). Moreover, there are over 250 blood group and 12 human platelet antigens assigned to one of the blood group or HPA systems, respectively. A system is defined by a gene or group of genes at a specific locus of the human genome. The alleles or genotype of a person for each blood group or HPA system represent the unique nucleotide gene sequences that express specific blood group or platelet antigens (for a review see Denomme, G. et al.,

Approaches to Blood Group Molecular Genotyping and Its Applications: in Stowell, C. and Dzik W., editors; *Emerging Technologies in Transfusion Medicine*, AABB 2003, Ch 4).

A blood group or HPA system maps to a specific region of the human genome, termed a locus. Nearly all blood group or HPAs can be identified by the presence of its unique nucleotide sequence, termed an 'allele', at the locus of interest. Every person has two alleles for any given autosomal gene. Some individuals are homozygotes for a specific allele, i.e. they have two identical alleles, while others are heterozygotes for a specific allele, i.e. they have two different alleles. By definition, alleles that represent different blood group or HPAs differ by at least one nucleotide; sometimes they differ by several nucleotides. For example, a deoxythymidine (T) or a deoxycytidine (C) nucleotide can be found at cDNA position 196 of the glycoprotein IIIa (GP3A) gene that expresses the HPA-1 (Newman P.J. et al., J Clin Invest 1989;83:;1778). The allele containing the deoxthymidine nucleotide expresses the HPA-1a antigen and the allele containing the deoxycytidine nucleotide expresses the HPA-1b antigen. We refer to the T/C nucleotide difference between the two alleles as a single nucleotide polymorphism (SNP).

Blood group alleles for a given blood group system represent genetic variations of the same gene. For example, the ABO blood group system has 3 common alleles, that confer 6 genotypes within this blood group system. Moreover, many alleles within a blood group system express different blood group 'antigens', that is to say, dependent on the allelic genotype the corresponding antigenic phenotype is accordingly expressed. Alleles differ in their nucleotide sequence, and the difference between one

allele and another, usually within a single blood group system, may be one single nucleotide variation. Therefore, two alleles can differ by one nucleotide, i.e. a SNP and represent a co-dominant bi-allelic system. Alternatively, alleles can differ by a few to several dispersed nucleotides, or by a stretch of nucleotides, any one of which can be used to identify the alleles. Regardless of whether the variations in the nucleotide are due to single or multiple nucleotide differences, the phenotype associated with a specific genotype (the specific nucleotide sequence) will result in the expression of a specific blood group or platelet antigen on the red cell or platelet surface, respectively.

Normally, all blood donations are blood grouped for ABO and RhD. However, sometimes a previously transfused recipient will require more blood that is antigen-matched with one of their own antigens because they have made antibodies to a different blood group or platelet antigen. The gold standard in the industry is to 'phenotype' blood for the presence of specific blood group and platelet antigens using government regulated antisera (antibodies) performed by single-test methods or by an automated platform, which is a cost ineffective method for a blood collection facility that routinely performs tests on a high volume basis.

Blood group phenotypes are presently determined using commercially available government-regulated serological reagents and human red cells. These known tests rely on the principle of antibody binding and red cell agglutination to identify clinically important blood group phenotypes. The presently known tests were originally devised some 60 years ago and today require the use of

government regulated (for example, Health Canada) approved serological reagents. Some of the tests being employed today have been automated (for example, ABO and Rh typing) while some have been semi-automated (for example, RhC/c and RhE/e). However, many of the presently used tests are performed manually by highly-trained laboratory technologists and are done on a test-by-test basis. In other words, a technologist must perform four separate tests to determine, for example, the Fy^a, Fy^b, Jk^a and Jk^b phenotype of a single blood donation. Essentially, the current tests which employ government-approved reagents in a manual, single-test driven method are a very cost ineffective method for a blood collection facility that is often required to perform such tests on a high volume basis.

In an effort to reduce costs, a blood collection facility will often use non-regulated antisera to 'screen' blood donations for important blood group phenotypes and then confirm the phenotype with the regulated antisera. However, since much of the blood is sent to hospitals within 24-48 hours after collection, manual blood group phenotyping cannot meet the short turn-around time required to provide the end user with the information required before blood must be shipped. Therefore, hospital blood banks must perform their own tests on the blood that they have in their inventories. It would be advantageous to provide a cost effective blood screening method that would provide quick and reliable results relating to the clinically important blood group phenotypes.

The prior art uses two basic techniques to detect SNPs; polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)(Chaudhuri A., et al. 1995;85:615),

and sequence specific primer (SSP)-PCR (McFarland J.G. et al., Blood 1991;78:2276). For PCR-RFLP analysis, restriction enzymes are used to digest PCR amplified genomic DNA fragments. In brief, DNA is extracted from nucleated blood cells manually for each blood sample to be analyzed. The PCR is set up manually; a separate PCR is performed on each sample for each SNP of interest. The PCR amplified fragments are digested with a specific restriction enzyme and the digested products are separated on a gel. The pattern of digested DNA fragments viewed from the gel predicts the presence or absence of either nucleotide of a SNP of interest. In SSP-PCR, two PCRs are set up in separate tubes for each SNP of interest. One tube contains a universal primer and a primer with a sequence that is specific to detect one nucleotide of a SNP. The other tube contains the same universal primer and a primer specific for the other nucleotide of a SNP. Prior art has used two pair or three pair PCR to analyze a nucleotide for a given SNP, with at least one pair acting as an internal control to ensure DNA is available for PCR amplification. The prior art does not provide the use of multiple DNA sequences as primer pairs that work simultaneously on a single sample. Moreover, the prior art does not employ novel DNA sequences to detect blood group SNPs in an automated high-throughput fashion.

St-Louis M., *et al.* (*Transfusion* 2003;43:11126-32) have used allele-specific PCR-ELISA to detect blood group SNPs, wherein some of the PCR primers were publicly known and all primers were labeled with digoxigenin; SNPs were detected by oligonucleotide hybridization using solid-phase microplate wells coated with individual blood group-specific complementary oligonucleotides. An abstract by Buffleir E. *et al.* (*Transfusion* 2003;43:92A) outlines a

combined HPA-1 and HPA-5 genotyping method that uses biotin labeled PCR-amplified targets and allele specific oligonucleotide probes arrayed on the bottom of 96 well microplates. Specific hybridization is detected with the use of an enzyme conjugate which produces a specific colourimetric signal. An array of several oligonucleotides reportedly can be used to detect HPA SNPs. The publications, cited above, do not use multiplex PCR primers, nor do they use extension probes, and rely on a less sensitive and more error-prone allele-specific hybridization to detect the SNPs. There are a few other publications that refer to the multiplex PCR amplification of the RHD gene alone, or together with sex determination, or with internal control primers designed to confirm the presence of DNA in various blood group PCR applications. United States Patent 5,723,293 describes a diagnostic method and kit for determining Rh blood group genotypes, wherein there is provided a method for directly determining D and associated CcEe genotypes using restriction fragment length polymorphisms (RFLPs) for diagnosis. USP 5,804,379 describes a diagnostic method and kit for determining Kell blood group genotype, wherein there is provided a method for determining the K1/K2 genotype using RFLPs for diagnosis. USP 5,780,229 provides polynucleotides for determining the Pen polymorphism of human platelet membrane glycoprotein IIIa, and generally describes diagnostic and therapeutic uses relating to the "Pen" human platelet polymorphism (HPA-4) and differs from the teachings of the present invention. United States patent application 20020098528 describes methods and apparatus for blood typing with optical bio-disc, and essentially describes a method for determining the ABO blood cell type

of an individual with optical bio-discs and a disc-reading apparatus.

In the SSP-PCR application by St. Louis *et al.* (*Transfusion* 2003;43:1126), two PCR primer pairs are set up, each in a separate well, to detect the nucleotides of a SNP of interest. For example, one primer pair containing a universal primer and a sequence specific primer is set up in a tube to detect a nucleotide of a SNP. Another primer pair containing the same universal and another sequence specific primer is set up in another tube to detect the alternate nucleotide for the same SNP. In addition, each tube includes a primer pair that detects a universal sequence contained in all human DNA. Contained in the PCR tube is digoxigenin-dUTP that is incorporated into the amplified DNA fragment if the sequence specific primer detects the appropriate nucleotide of an SNP. For the detection phase, one of each primer pair contains the chemical tag biotin, which is used to capture the DNA amplified fragment in sets of microtitre wells containing streptavidin. An optical colorimetric assay is used to detect the presence of digoxigenin-dUTP in each of the wells; anti-digoxigenin peroxidase conjugated antibody detects the presence of digoxigenin dUTP and the peroxidase can convert a substrate added to the well into a colored end product. Therefore, the presence of a nucleotide of a SNP is detected by the presence of a color in the microtitre well. Such assays are routinely designed in a 96-well microtitre plate format to facilitate semi-automation. The colorimetric results are evaluated by the operator to determine the presence or absence of the nucleotides for a SNP. The deficiencies of these test systems are the use of a single PCR reaction for each nucleotide of a given nucleotide of each SNP, and the

pooling of samples prior to the detection phase and manual post-analyte data analysis.

No prior art has used a multiple, or 12, primer pair multiplexed PCR that successfully works in a single tube, nor has prior art employed novel DNA sequences as probes to detect both nucleotides of a plurality of blood group and HPA genotypes simultaneously, such as the detection of all 12 blood group and HPA SNPs in these mixtures using an automated high-throughput platform.

Accordingly, there is a need for a high-throughput automated multiple blood-group associated SNP analysis of genomic DNA that is capable of rapidly and accurately determining the genotypes and associated phenotypes of a plurality of blood group systems in a single test sample.

SUMMARY OF THE INVENTION

The present invention provides a method of detecting the presence or absence of nucleotides relating to various SNPs for the determination of a specific genotype and accordingly the inferred phenotype. More specifically, the present invention allows for the detection of the presence or absence of two nucleotides of a plurality of different SNPs, and more preferably of the 12 SNPs in a preferred embodiment of the present invention.

The present invention accordingly provides an automated, or robotic, high-throughput 'screening' tool for blood group and platelet antigens by evaluating the alleles of the genes that express these antigens on red cells and platelets, respectively. This is done by identifying the unique nucleotides associated with the specific alleles that occupy the gene locus using a testing platform, which requires novel and specific compounds that we designed.

Our robotic high-throughput platform provides important blood group and HPA genotype information within 24 hours from the start of the test. We identified the alleles of blood group antigens for; RhD, RhC, Rhc, RhE, Rhe, S, s, Duffy $(Fy)^a$, Fy^b, K, k, Kp^a, Kp^b, Diego $(Di)^a$, Di^b, Kidd $(Jk)^a$, Jk^b, and the platelet antigens, Human Platelet Antigen (HPA)-1a and HPA-1b, representing, but not limited to 19 of the most clinically important antigens in red cell and platelet transfusion. Additional genotyping tests for other clinically important blood group and platelet antigens may be developed, and are encompassed in the teachings of the present invention. When performed on all blood donations for all clinically important blood group and platelet antigens, our invention will provide a comprehensive database to select and confirm the antigens when required using government regulated antisera. The use of this platform as a screening tool will lessen the number of costly government regulated tests to be done by the collection facility and end user (the hospital blood bank), and meet the demand of antigen-matched blood for specific transfusion recipients.

The invention discloses a method for DNA-based blood group genotyping for clinically important blood group and platelet antigens. The technology uses an ultra high-throughput multiplex PCR design to detect specific SNPs that represent clinically important blood group antigens: RhD, RhC, Rhc, RhE, Rhe, S, s, Duffy $(Fy)^a$, Fy^b, K, k, Kp^a, Kp^b, Diego $(Di)^a$, Di^b, Kidd $(Jk)^a$, Jk^b, and the platelet antigens, Human Platelet Antigen (HPA)-1a and HPA-1b. It should be noted however that the present invention is not limited to the detection of SNPs for only the SNPs listed, but additionally comprises the detection of SNPs for all blood group and platelet antigens. The invention discloses

novel DNA sequences of PCR primers that are specifically designed to avoid inter-primer pair cross-reactions and post-PCR probes that make multiple analyses possible. The invention represents a novel approach to screening multiple blood group and HPA genotypes at once and addresses a clear need in the art for novel, rapid, cost-effective and reliable genotyping. This additionally replaces the use of expensive and difficult-to-obtain serological reagents, which can be reserved for use to confirm only the donors identified by the screening process.

More specifically, the present invention analyzes the HPA-1 GP3A mutation incorporated into our SNP assay, and the other blood group antigen SNPs in a method according to the present invention.

The invention addresses the need for an automated, accurate, rapid and cost-effective approach to the identification of multiple blood group antigens. The multiplex SNP assay design and automated genotyping platform allows one trained research technician to identify a plurality of blood group alleles, and more specifically, 19 blood group alleles, overnight on 372 to 2232 individual blood samples. In one application of the present invention, the multiplex PCR and SNP detection platform analyzed the nucleotides of 12 SNPs overnight on 372 individual blood samples. The cost using current standard blood group serology for 372 samples is estimated at CDN$99,500, which reflects a reagent cost of CDN$54,000 (excluding new capital equipment investments) and an operator cost of CDN$45,500 to analyse each of the antigens by Gel Card technology (n=5), immediate spin tube test (n=2), indirect antiglobulin tube test (n=8), and platelet GTI® test (n=1). Approximate 10 to 15 fold cost savings are obtained in the

simultaneous DNA-based determination of these blood group alleles. It should be noted that the present invention is not limited to the detection of only 12 SNPs, and may be optimally used for the detection a plurality of SNPs for potentially all blood group and platelet alleles. Accordingly, the products, methods, platform and teachings of the present invention can detect all blood group and HPA SNP variations on a great number of samples, such as 744 samples overnight, as further described below.

The present invention overcomes the deficiencies of the prior art because the entire test, i.e. all steps of the method of the present invention, from PCR to computation analyses can be automated and multiplexed so that the nucleotides of a plurality of SNPs, and more preferably, the 12 SNPs of the present invention, can be identified simultaneously. This automated multiplex high throughput analysis can meet the demand of testing hundreds of blood samples, and the turn-around time of less than 24 hours, to provide valuable information to a blood collection facility before blood is shipped to the end user. This platform has the advantage over existing technology in that it reduces operator handling error. In addition, there are significant cost reductions compared with the current government-regulated serological analysis. It should be noted that present prior art technologies relating to PCR-RFLP and SSP-PCR for blood group and platelet antigens are not routinely used since they are no more cost efficient than serology. The present invention overcomes the deficiencies of the prior art and fulfils an important need in the present art for the automated, accurate, rapid and cost-effective identification of multiple blood group and HPA SNPs.

The invention provides the opportunity to screen all blood donors to obtain a daily or 'live' repository of the genotypes or combinations of genotypes currently available for specific transfusion needs. Accordingly, the present invention fulfills a need relating to the collection and antigen screening of blood and blood products.

For convenience, some terms employed in the present specification are noted below. Unless defined otherwise, all technical and scientific terms used herein have the meanings commonly understood by one of ordinary skill in the present art.

The present invention provides a method or screening assay for the determination of blood genotypes of the various blood group and HPA systems through the ultra high throughput multiplex PCR analysis of SNPs in an automated platform (Petrick J. Vox Sang 2001;80:1). A platform, as referred to herein, refers to a system of machine(s) and protocol(s) capable of analyzing multiplex PCR amplified SNPs, wherein said platform is not limited to, but may comprise the GenomeLab SNPStream (Beckman Coulter Inc., Fullerton, CA), the SNPStream™ UHT (Orchid BioSciences, Princeton, NJ), the SNPStream™ 25K (Orchid BioSciences, Princeton, NJ), the MALDI-TOF/Mass-Spectrophotometer Spectro CHIP (Sequenom, San Diego, CA), and the Gene Chip Microarray (Affymetrix, Inc., Santa Clara, CA), Nano Chip (Nanogen, San Diego, CA) and the Random Ordered Bead Arrays (Illumina, Inc., San Diego, CA) or any other system, machine or protocol capable of analyzing multiplex PCR amplified SNPs. Accordingly, the present invention provides a platform, or system and protocols, for the evaluation and detection of SNPs, for the purpose of typing (determining the genotype and corresponding

phenotype) blood group and platelet, preferably, human platelet antigen (HPA) SNP analysis. A preferred platform that can be used in accordance with the present invention is the Orchid SNP-IT system for HLA typing (Orchid Bioscience, Princeton, NJ), wherein a preferred embodiment of the present invention comprises the use of the primer pairs of Table 1 for the specific oligonucleotide primer extension of blood group and platelet, preferably, human platelet antigen (HPA) SNPs, and the probes of Table 2 for the specific hybridization thereof, and the simultaneous analysis of the absence or presence of a plurality of blood group and platelet, preferably, human platelet antigen (HPA) SNPs using a platform as described herein, or using any SNP analysis system capable of detecting multiplex PCR amplified SNPs.

For the purposes of the present disclosure, SNPs, may refer to any blood group and HPA SNPs, and more preferably refers to any of the SNPs specified in Table 1, or any other known blood group or HPA SNPs or single nucleotide changes including, but not limited to, nucleotide substitutions, deletions, insertions or inversions, that can be defined as a blood group or HPA SNP due to nucleotide differences at the specified position in a gene sequence.

Ultra high throughput (UHT) refers to the implementation of the platform in a rapid and optimized form, that is to say, through the analysis of multiple SNPs. That is to say, UHT analysis refers to the rapid and simultaneous evaluation of a plurality of samples for a plurality of markers, in this case SNPs. For example, the analysis of 12 SNPs (equivalent to 12 C and 12 T nucleotides) for 372 samples, would result in the

generation of 8928 (i.e.2x12x372) determinations that are analysed, an evaluation that far exceeds the number of evaluation points possible with manual or automated serological methods.

Phenotype in the context of red cell blood group and Human Platelet Antigen (HPA) refers to the expressed moiety of an allele for a given gene, and is also referred to in this document as 'antigen'. Genotype refers to the two alleles of an autosomal gene that occupy a given locus or alternatively to either one or two alleles of an X-linked gene that occupies a given locus.

Antigen refers to a red cell or platelet membrane carbohydrate, protein or glycoprotein that is expressed as a polymorphic structure among the human population, that is to say a moiety that is immunogenic in another animal, or human, due differences in its amino acid or carbohydrate composition. Blood group or red cell, or HPA or platelet antigen refers to a moiety expressed on red cells or platelets that has been assigned a blood group or Human Platelet Antigen (HPA) designation, or provisional or workshop designation. The present invention comprises a method and for the determination of the antigen genotype and corresponding phenotype of any blood group or red cell, or HPA or platelet antigen using multiplex PCR SNP analysis. The following two tables (Table A and Table B) list most of the known human blood group and platelet antigens. Many of the antigens can be identified by their unique nucleotide sequence.

Table A Human Red Cell Blood Group Systems

ISBT Name (ISBT Number)	Chromosome Location	Gene Name ISGN (ISBT)	Component Name (CD Number)	Associated Blood Group Antigens
ABO (001)	9q34.2	*ABO (ABO)*	Carbohydrate	A, B, A, B, A1
MNS (002)	4q28.2-q31.1	*GYPA (MNS)* *GYPB (MNS)*	GPA (CD235a) GPB (CD235b)	M, N, Vw, S, s, U, He + 36 more
P (003)	22q11.2-qter	*P1 (P1)*	Carbohydrate	P1
Rh (004)	1p36.13-p34.3	*RHD (RH)* *RHCE (RH)*	RhD (CD240D) RhCE (CD240CE)	D, G, Tar C, E, c, e, V, Rh17 + 39 more
Lutheran (005)	19q13.2	*LU (LU)*	Lutheran glyco-protein B-CAM (CD239)	Lu^a, Lu^b, Lu3, Lu4, Au^a, Au^b + 13 more
Kell (006)	7q33	*KEL (KEL)*	Kell glycoprotein (CD258)	K, k, Kp^a, Kp^b, Ku, Js^a, Js^b + 17 more
Lewis (007)	19p13.3	*FUT3 (LE)*	Carbohydrate Adsorbed form plasma	Le^a, Le^b, Le^{ab}, Le^{bh}, ALe^b, BLe^b
Duffy (008)	1q22-q23	*DARC (FY)*	Fy glycoprotein (CD234)	Fy^a, Fy^b, Fy3, Fy4, Fy5, Fy6
Kidd (009)	18q11-q12	*SLC14A1 (JK)*	Kidd glycoprotein	Jk^a, Jk^b, Jk3
Diego (010)	17q21-q22	*SLC4A1 (DI)*	Band 3, AE1 (CD233)	Di^a, Di^b, Wr^a, Wr^b, Wd^a, Rb^a +14 more
Yt (011)	7q22	*ACHE (YT)*	Acetyl-cholinesterase	Yt^a, Yt^b
Xg (012)	Xp22.32	*XG (XG)* *MIC2*	Xg^a glycoprotein CD99	Xg^a CD99
Scianna (013)	1p34	*ERMAP (SC)*	ERMAP	Sc1, Sc2, Sc3, Rd

ISBT Name (ISBT Number)	Chromosome Location	Gene Name ISGN (ISBT)	Component Name (CD Number)	Associated Blood Group Antigens
Dombrock (014)	12p13.2-p12.1	*DO (DO)*	Do glycoprotein; ART 4	Do^a, Do^b, Gy^a, Hy, Jo^a
Colton (015)	7p14	*AQP1 (CO)*	Channel-forming integral protein	Co^a, Co^b, Co3
Landsteiner-Wiener (016)	19p13.3	*LW (LW)*	LW glycoprotein (ICAM-4) (CD242)	LW^a, LW^{ab}, LW^b
Chido/ Rodgers (017)	6p21.3	*C4B, C4A (CH/RG)*	C4B, C4A	CH1, CH2, Rg1 + 6 more
Hh (018)	19q13.3	*FUT1 (H)*	Carbohydrate (CD173)	H
Kx (019)	Xp21.1	*XK (XK)*	Xk glycoprotein	Kx
Gerbich (020)	2q14-q21	*GYPC (GE)*	GPC GPD (CD236)	Ge3, Ge4, Wb, Ls^a, Dh^a Ge2, Ge3, An^a
Cromer (021)	1q32	*DAF (CROM)*	DAF (CD55)	Cr^a, Tc^a, Tc^b, Tc^c, Dr^a, Es^a, IFC, WES^a,WES^b, UMC, GUTI
Knops (022)	1q32	*CR1 (KN)*	CR1 (CD35)	Kn^a, Kn^b, McC^a, Sl^a, Yk^a
Indian (023)	11p13	*CD44 (IN)*	Hermes antigen (CD44)	In^a, In^b
OK (024)	19pter-p13.2	*CD147 (OK)*	Neurothelin, basogin (CD147)	Ok^a
RAPH (025)	11p15.5	*MER2 (MER2)*	Not defined	MER2
JMH (026)	15q22.3-q23	*SEMA-L (JMH)*	H-Sema-L (CD108)	JMH
I (027)	6p24	*CGNT2 (IGNT)*	Carbohydrate	I

ISBT Name (ISBT Number)	Chromosome Location	Gene Name ISGN (ISBT)	Component Name (CD Number)	Associated Blood Group Antigens
Globoside (028)	3q25	*B3GALT3(βGalNAcT1)*	Carbohydrate (Gb_4, globoside)	P
GIL (029)	9p13	*AQP3 (GIL)*	AQP3	GIL

ISGN= International Society for Gene Nomenclature

Table B Human Platelet Antigen Systems

System	Gene Name	Chromosome Location	Component Name (CD)	Associated Antigens
HPA-1	*GP3A*	17q21.32	Integrin β3 (CD61)	$PI^{A1/2}$
HPA-2	*GP1BA*	17pter-p12	Glycoprotein Ibα (CD42b)	$Ko^{a/b}$
HPA-3	*GP2B*	17q21.32	Integrin α2b (CD41)	$Bak^{a/b}$
HPA-4	*GP3A*	17q21.32	Integrin β3 (CD61)	$Pen^{a/b}$
HPA-5	*GP1A*	5q23-q31	Integrin α2 (CD49b)	$Br^{a/b}$
HPA-6w	*GP3A*	17q21.32	Integrin β3 (CD61)	Ca^a/Tu^a
HPA-7w	*GP3A*	17q21.32	Integrin β3 (CD61)	Mo^a
HPA-8w	*GP3A*	17q21.32	Integrin β3 (CD61)	Sr^a
HPA-9w	*GP2B*	17q21.32	Integrin α2b (CD41)	Max^a
HPA-10w	*GP3A*	17q21.32	Integrin β3 (CD61)	La^a
HPA-11w	*GP3A*	17q21.32	Integrin β3 (CD61)	Gro^a
HPA-12w	*GP1BB*	22q11.2	Glycoprotein Ibβ (CD42c)	Ly^a
HPA-13w	*GP1A*	5q23-q31	Integrin α2 (CD49b)	Sit^a
HPA-	*GP3A*	17q21.32	Integrin β3 (CD61)	Oe^a

System	Gene Name	Chromosome Location	Component Name (CD)	Associated Antigens
14w				
HPA-15	*AF410459*	6q13	GPI-linked GP (CD109)	$Gov^{a/b}$
HPA-16w	*GP3A*	17q21.32	Integrin β3 (CD61)	Duv^{a}
?	*GPV*	?	Glycoprotein V	PI^{T}
?	*GPIV*	7q11.2	Glycoprotein IV (CD36)	Vis^{a}/Nak^{a}

Note: HPA numbers on the left ending with a 'w' represent ISBT workshop designations and are tentative HPA systems.

A single nucleotide polymorphism (SNP) refers to any blood group or HPA allele that defines a specific red cell or platelet antigen by virtue of its unique nucleotide sequence as defined in Garratty et al. Transfusion 2000;40:477 and as updated from time-to-time by the International Society of Blood Transfusion.

It is understood that the presently disclosed subject matter is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as these can vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the presently disclosed subject matter.

Unless defined otherwise, all technical and scientific terms used herein are intended to have their meanings as understood by one skilled in the present art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the presently disclosed subject matter, the preferred embodiments, methods, devices and materials described.

It is also understood that the articles 'a' and 'an' are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. Accordingly, 'an element' means one element or more than one element.

Our novel platform simultaneously performs automated multiple blood group-associated SNP analyses using genomic DNA and the *Thermus aquaticus* polymerase chain reaction (PCR) to infer the presence of specific blood group genotypes. This automated high-throughput platform has particular application in the blood donation industry since it represents a novel screening tool for the expression of blood group antigens or phenotypes.

Our platform provides important genotypic information within 24 hours of donation. When performed on all blood donations for all important blood group phenotypes, our invention will provide a comprehensive database to select and confirm blood group phenotypes using government regulated antisera. The use of this platform as a screening tool will lessen the number of regulated blood group phenotype tests done by the collection facility and end user, and meet the end user demand for antigen-matched blood for transfusion recipients.

Unique to this invention is the assay design for the simultaneous identification of a plurality of blood group or HPA alleles. The present invention provides novel assay for the simultaneous identification of a plurality of blood group or HPA alleles, and more preferably of 19 blood group alleles using a plurality of SNPs, and more preferably, 12 SNPs. In one embodiment, the genotyping platform queries genetic variants using multiplexed single nucleotide primer extension coupled with two-laser fluorescence detection and

software for automated genotype calling. Each of the relevant gene regions are PCR amplified from purified genomic DNA in a single reaction using the following oligonucleotide primer designs:

Gene	**Primer**	**Sequence (5' - 3')**
RHD Exon 4	RHDe4S	AGACAAACTGGGTATCGTTGC
	RHDe4A	ATCTACGTGTTCGCAGCCT
RHD Exon 9	RHDe9S	CCAAACCTTTTAACATTAAATTATGC
	RHDe9A	TTGGTCATCAAAATATTTAGCCTC
RHCE Exon 2	RHCEe2S	TGTGCAGTGGGCAATCCT
	RHCEe2A	CCACCATCCCAATACCTG
RHCE Exon 5	RHCEe5S	AACCACCCTCTCTGGCCC
	RHCEe5A	ATAGTAGGTGTTGAACATGGCAT
GYPB Exon 4	GYPBe4S	ACATGTCTTTCTTATTTGGACTTAC
	GYPBe4A	TTTGTCAAATATTAACATACCTGGTAC
KEL Exon 6	KELe6S	TCTCTCTCCTTTAAAGCTTGGA
	KELe6A	AGAGGCAGGATGAGGTCC
KEL Exon 8	KELe8S	AGCAAGGTGCAAGAACACT
	KELe8A	AGAGCTTGCCCTGTGCCC
FY Promoter	FYproS	TGTCCCTGCCCAGAACCT
	FYproA	AGACAGAAGGGCTGGGAC
FY Exon 2	FYe2S	AGTGCAGAGTCATCCAGCA
	FYe2A	TTCGAAGATGTATGGAATTCTTC
JK Exon 9	JKe9S	CATGAACATTCCTCCCATTG
	JKe9A	TTTAGTCCTGAGTTCTGACCCC
DI Exon 18	DIe19S	ATCCAGATCATCTGCCTGG
	DIe19A	CGGCACAGTGAGGATGAG

GP3A GP3Ae3S ATTCTGGGGCACAGTTATCC

GP3Ae3A ATAGTTCTGATTGCTGGACTTCTC

The above primer pairs comprise the corresponding forward and reverse primers, and may be referred to herein as SEQ ID NOs 1-24.

Multiplexed single nucleotide primer extension is performed using the following 5′ tagged extension primers:

RHD Exon 4 **GTGATTCTGTACGTGTCGCC**GTCTGATCTTTATCCTCCGTTCCCT

RHD Exon 9 **GCGGTAGGTTCCCGACATAT**TTTAAACAGGTTTGCTCCTAAATCT

RHCE Exon 2 **GGATGGCGTTCCGTCCTATT**GGACGGCTTCCTGAGCCAGTTCCCT

RHCE Exon 5 **CGACTGTAGGTGCGTAACTC**GATGTTCTGGCCAAGTGTCAACTCT

GYPB Exon 4 **AGGGTCTCTACGCTGACGAT***TTGAAATTTTGCTTTATAGGAGAAA*

KEL Exon 6 **AGCGATCTGCGAGACCGTAT**TGGACTTCCTTAAACTTTAACCGAA

KEL Exon 8 **AGATAGAGTCGATGCCAGCT**TTCCTTGTCAATCTCCATCACTTCA

FY Promoter **GACCTGGGTGTCGATACCTA**GGCCCTCATTAGTCCTTGGCTCTTA

FY Exon 2 **ACGCACGTCCACGGTGATTT**GGGGGCAGCTGCTTCCAGGTTGGCA

JK Exon 9 **CGTGCCGCTCGTGATAGAAT**AAACCCCAGAGTCCAAAGTAGATGT

DI Exon 19 **GGCTATGATTCGCAATGCTT**GTGCTGTGGGTGGTGAAGTCCACGC

GP3A Exon 3 **AGAGCGAGTGACGCATAC**TTGGGCTCCTGTCTTACAXGCCCTGCCTC

The above probes may be referred to herein as SEQ ID NOs 25-36. The DNA bases are represented by their single letter equivalents (A,C,G or T) and the letter X represents a C3 (phosphoramidite) spacer between the two adjacent DNA bases.

In this embodiment, the 12 bolded nucleotides in the 5′ region of the extension probes are hybridized to a complementary DNA sequence that has been micro-arrayed onto

microplates so that specific blood group SNPs are individually identified and reported.

Proof of principle experiments have been performed using 372 consent qualified samples (please refer to Appendix A). Collection of serological data for samples has been constant and the success rates based upon the expected allele frequencies have been performed.

In the preceding example, one preferred embodiment has been described. However, it should be obvious to one skilled in the art that other methodologies and/or technologies for SNP identification could be used, providing that the novel DNA sequences disclosed above are also used.

The teachings and method of the present invention are superior to the teachings of the prior art for a number of reasons, one of which is that the complete method of the present invention, from DNA extraction to result computation analyses can be automated and multiplexed so that many SNPs can be determined simultaneously. This automated multiplex high throughput analysis can meet the demand (hundreds of blood donations can be tested) and the turn-around time (< 24 hours) to collate and provide valuable information to the blood collection facility before blood is shipped to the end user. This platform and method has the further advantage over existing technology in that it reduces operator handling error.

In addition, there are significant cost reductions compared with the current technology. The invention addresses the need for an automated, accurate, rapid and cost-effective approach to the identification of multiple blood group SNPs. According to an embodiment, a multiplex

SNP assay of the present invention detected 12 SNPs overnight on 372 individual blood samples. In accordance with the teachings of the present invention, the platform, products and methods of the present invention can detect all SNP variations for all blood group antigens, for example, as shown below on 744 samples.

BRIEF DESCRIPTION OF THE DRAWINGS

Further features and advantages of the present invention will become apparent from the following detailed description, taken in combination with the appended drawings, in which:

Fig. 1 A computer screen display of a typical UHT SNP scatter plot to sort the fluorescence of a C/T SNP analysis of GP3A Exon 3 for HPA-1a/b genotyping.

Fig. 2 Representative samples of GP3A Exon 3 HPA-1a/b) genotyping by manual PCR-RFLP analysis using *Msp*I restriction enzyme analysis (A) and the tabulated comparative results with the UHT SNP analysis (B).

Fig. 3 Representative samples JK genotyped by manual PCR-RFLP analysis using *Mnl*I (A) and the tabulated comparative results with the UHT SNP analysis (B).

Fig. 4 A-L Computer screen displays of typical UHT SNP scatter plots to sort the fluorescence of a C/T SNP for various blood group and HPA genotypes.

Appendix A provides a tabulated summary of the multiplex SNP assay detection of 12 possible SNPs on 372 individual blood samples.

It will be noted that throughout the appended drawings, like features are identified by like reference numerals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

RBC and platelet (Plt) alloimmunization requires antigen-matched blood to avoid adverse transfusion reactions. Some blood collection facilities use unregulated Abs to reduce the cost of mass screening, and later confirm the phenotype with government approved reagents. Alternatively, RBC and Plt antigens can be screened by virtue of their associated single nucleotide polymorphisms (SNPs). The present invention provides a multiplex PCR-oligonucleotide extension assay using the GenomeLab SNPStream platform, or any other SNP analysis system, to genotype blood for a plurality of common antigen-associated SNPs, including but not limited to: RhD (2), RhC/c, RhE/e, S/s, K/k, $Kp^{a/b}$, Fya/b, FY0, $Jk^{a/b}$, $Di^{a/b}$, and HPA-1a/b. According to one example of the present invention, a total of 372 samples were analysed for 12 SNPs overnight. Individual SNP pass rates varied from 98-100% for 11 of 12 SNPs. Of the Rh-pos, 98.6% were correctly identified. Six of 66 Rh-neg (9%) were typed as RHD-pos; 5 of 6 were subsequently demonstrated to contain an non-RHDψ gene by SSP-PCR. Eleven of 12 R1R1 and 1 of 1 r"r were correctly identified. HPA-1b was identified in 4, which was confirmed by PCR-RFLP (n=4) and serology (n=1). PCR-RFLP on selected samples ($n<20$) for K/k, $Fy^{a/b}$, and $Jk^{a/b}$ were 100% concordant. Confirmation of some of the results is provided. The platform has the capacity to genotype thousands of samples per day for all SNP variations. The suite of SNPs can provide collection facilities with real-time genotypic data for all donors at an annual cost

(excluding RhD) estimated to equal the current cost of phenotyping 5-10% of the donors.

METHODS and REAGENTS

Methodology Specific to the Invention.

We have designed a novel blood group and HPA SNP and detection system that employ the use of two sets of novel compounds (reagents) that are specifically designed to work in a multiplex format.

In brief, genomic DNA is harvested the salting out procedure using the Qiagen (Qiagen Inc. Valencia, CA) Blood DNA Isolation Kit. Our invention can use any good quality DNA harvested by any one of a variety of methods. For the multiplex PCR, the DNA regions containing all 12 SNPs of interest were PCR-amplified in a single reaction well. Tables 1 and 2 outline the novel PCR primers and extension probes, respectively, used in the assay. Note that the concentration of the various reagents may be adjusted to optimize DNA amplification, and is dependent on but is not limited to: the concentration and quality of the genomic DNA, the concentration of the PCR primers or the type of thermal cycler used for the PCR.

Our current genotyping technology identifies SNPs using single base-pair primer extension using the novel products and protocols of the present invention. In brief, the genomic region surrounding the SNP of interest is PCR-amplified as described above, preferably using one or more, or all of the primer pairs of Table 1. Then, the amplified DNA fragments are used as a template for DNA hybridization using one or more or all the corresponding novel probes of Table 2, and single nucleotide extension (synthesis) based on the nucleotide present at each of the specific SNP

sites. The PCR primers pairs in Table 1 represent sequences complementary to DNA regions containing SNPs of interest; of which the exact sequences of each primer pair and mixture of primer pairs have been specifically optimized to amplify genomic DNA of interest as a mixture of 12 primer pairs. Although noted above, Table 2 further summarizes 12 novel extension primers specifically used together to detect the nucleotides of blood group and platelet antigen or HPA SNPs, simultaneously. The extension primers represent a group of 12 novel nucleotide sequences, of which each are a combination of: 1) a unique 5′ region necessary to direct hybridization to a micro-arrayed tag located in a specific spot in each microplate well, and 2) a 3′ region complementary to and adjacent to a SNP of a PCR-amplified DNA region containing the SNP of interest.

Table 1. The PCR primers used in the 12-pair multiplex PCR format for multiple SNP detection.

Antigen	SNP	Primer Name	Sequence 5′-3′	Product Target	Size (bp)
RhD/RhCE	C/T	RHDe4S	AGACAAACTGGGTATCGTTGC	RHD Exon 4	111
		RHDe4A	ATCTACGTGTTCGCAGCCT		
RhD/RhCE	A/G	RHDe9S	CCAAACCTTTTAACATTAAATTATGC	RHD Exon 9	98
		RHDe9A	TTGGTCATCAAAATATTTAGCCTC		
RhC/Rhc	T/C	RHCEe2S	TGTGCAGTGGGCAATCCT	RHCE Exon 2	90
		RHCEe2A	CCACCATCCCAATACCTG		
RhE/Rhe	C/G	RHCEe5S	AACCACCCTCTCTGGCCC	RHCE Exon 5	107
		RHCEe5A	ATAGTAGGTGTTGAACATGGCAT		
GYPBS/GYPBs	T/C	GYPBe3S	ACATGTCTTTCTTATTTGGACTTAC	GPYB Exon 4	103
		GYPBe3A	TTTGTCAAATATTAACATACCTGGTAC		
K/k	T/C	KELe6S	TCTCTCTCCTTTAAAGCTTGGA	KEL Exon 6	142
		KELe6A	AGAGGCAGGATGAGGTCC		
Kp^a/Kp^b	T/C	KELe8S	AGCAAGGTGCAAGAACACT	KEL Exon 8	100
		KELe8A	AGAGCTTGCCCTGTGCCC		
Fy/Fy0	T/C	FYproS	TGTCCCTGCCCAGAACCT	Duffy Promoter	90
		FYproA	AGACAGAAGGGCTGGGAC		
Fy^a/Fy^b	G/A	FYe2S	AGTGCAGAGTCATCCAGCA	Duffy Exon 2	122
		FYe2A	TTCGAAGATGTATGGAATTCTTC		
Jk^a/Jk^b	G/A	JKe9S	CATGAACATTCCTCCCATTG	Kidd Exon 9	130
		JKe9A	TTTAGTCCTGAGTTCTGACCCC		
Di^a/Di^b	T/C	DIe19S	ATCCAGATCATCTGCCTGG	Diego	90

		Die19A	CGGCACAGTGAGGATGAG	Exon 19	
HPA-1a/b	T/C	GP3Ae3S	ATTCTGGGGCACAGTTATCC	GP3A Exon 3	114
		GP3Ae3A	ATAGTTCTGATTGCTGGACTTCTC		

The above pimers may be referred to herein as SEQ ID NOs 1-24.

Table 1A. Additional Blood Group and Platelet Antigen SNPs for Clinically Relevant Antigens.

Antigen	SNP			Product Target	Size (bp)
A/O GalNAc/Del	G/T			ABO Exon 6	
A/B (GalNAc/Gal)	C/G			ABO Exon 7	
A/B (GalNAc/Gal)	G/A			ABO Exon 7	
A/B (GalNAc/Gal)	C/A			ABO Exon 7	
A/B (GalNAc/Gal)	G/C			ABO Exon 7	
M/N	G/A			MNS Exon 2	
M/N	T/G			MNS Exon 2	
MNS/MiI	C/T			MNS Exon 3	
RHD/Weak D Type 1	T/G			RHD Exon 6	
RHD/Weak D Type 2	G/C			RHD Exon 9	
RHD/Weak D Type 3	C/G			RHD Exon 1	
RHD/D nt602 Variants	C/G			RHD Exon 4	
RHD/'DAR' Variant	T/C			RHD Exon 7	
RHD/Weak D Type 5	C/A			RHD Exon 3	
RHD/D_{el}	G/A			RHD IVS3+1	
RHD/D_{el}	G/T			RHD Exon 6	
RHD/D_{el}	G/A			RHD Exon 9	
RHD/RHDψ nt506	A/T			RHD Exon 4	
RHCE/RhC	T/C			RHCE IVS2+1722	

RHCE/RhC	C/T			RHCE IVS2_1751	
RHCE/ VS variant	C/G			RHCE Exon 5	
Lu^a/Lu^b	A/G			LU Exon 3	
Au^a/Au^b	A/G			LU Exon 12	
Js^a/Js^b	C/T			KEL Exon 17	
Js/Js_{null}	G/T			JK IVS7+1	
FY/Fy^x	C/T			FY Exon 2	
FY/Fy^x	G/A			FY Exon 2	
Wr^a/Wr^b	A/G			DI Exon 16	
Yt^a/Yt^b	C/A			YT Exon 2	
Sc1/Sc2	G/A			SC Exon 3	
Do^a/Do^b (nt 378)	C/T			DO Exon 2	
Do^a/Do^b (nt 624)	T/C			DO Exon 2	
Do^a/Do^b (nt 793)	A/G			DO Exon 2	
Co^a/Co^b	C/T			CO Exon 1	
In^a/In^b	C/G			IN Exon 2	
Ok(a+)/Ok(a-)	G/A			OK Exon 4	
GIL/GIL_{null}	G/A			GIL IVS5	
HPA-2a/b	C/T			GP1BA Exon 2	
HPA-3a/b	T/G			GP2B Exon 26	
HPA-4a/b	G/A			GP3A Exon 4	
HPA-5a/b	G/A			GP1A Exon 13	
Gov^a/Gov^b	A/C			CD109 Exon 19	

Each antigen listed on the left represents a blood group or HPA genotype and the single nucleotide polymorphism (SNP). Some genotypes are evaluated using more than one SNP because they differ by more than one nucleotide. Each PCR primer pair consists of a sense (Primer Name ending in S) and antisense (Primer Name ending in A) oligonucleotide (Sequence 5'-3') designed to amplify the DNA region containing the SNP for the antigen of interest. The target region (Product Target) and the amplified fragment (Size (bp)) are shown on the right. Note that 12 SNPs are evaluated for 19 different blood group and platelet antigens because some antigens have more than one SNP. In some cases an A or G SNP is included since the complementary DNA strand can be evaluated as it will contain the T or C SNP of interest.

Table 2. Extension probes used to detect the nucleotides of blood group and HPA SNPs.

Name	Sequence 5'-3'
RHD Exon 4	**GTGATTCTGTACGTGTCGCC***GTCTGATCTTTATCCTCCGTTCCCT*
RHD Exon 9	**GCGGTAGGTTCCCGACATAT***TTTAAACAGGTTTGCTCCTAAATCT*
RHCE Exon 2	**GGATGGCGTTCCGTCCTATT***GGACGGCTTCCTGAGCCAGTTCCCT*
RHCE Exon 5	**CGACTGTAGGTGCGTAACTC***GATGTTCTGGCCAAGTGTCAACTCT*
GYPB Exon 4	**AGGGTCTCTACGCTGACGAT***TTGAAATTTTGCTTTATAGGAGAAA*
KEL Exon 6	**AGCGATCTGCGAGACCGTAT***TGGACTTCCTTAAACTTTAACCGAA*
KEL Exon 8	**AGATAGAGTCGATGCCAGCT***TTCCTTGTCAATCTCCATCACTTCA*
FY Promoter	**GACCTGGGTGTCGATACCTA***GGCCCTCATTAGTCCTTGGCTCTTA*
FY Exon 2	**ACGCACGTCCACGGTGATTT***GGGGGCAGCTGCTTCCAGGTTGGCA*
JK Exon 9	**CGTGCCGCTCGTGATAGAAT***AAACCCCAGAGTCCAAAGTAGATGT*
Di Exon 19	**GGCTATGATTCGCAATGCTT***GTGCTGTGGGTGGTGAAGTCCACGC*
GP3A Exon 3	**AGAGCGAGTGACGCATAC***TTGGGCTCCTGTCTTACAXGCCCTGCCTC*

The above probes may be referred to herein as SEQ ID NOs 25-36. The DNA bases are represented by their single letter equivalents (A,C,G or T) and the letter X represents a C3 (phosphoramidite) spacer between the two adjacent DNA bases.

The present invention also provides novel hybrid probes, wherein the preferred probes are listed in Table 2, but limited to said listing. Each extension probe is

designed in two parts: (1) the 5′ portion: the 5′ nucleotides indicated in boldface of the extension primer are complementary to unique and specific DNA sequences which are micro-arrayed onto the bottom of microplates in a specified location of each microplate well. Thus, the 5′ portion of the extension probes in table 2 represent, but are not limited to, 12 unique complementary sequences used together to identify the individual SNPs through hybridization to the micro-arrayed tags in the microplate wells. The 12 unique 5′ portions can be interchanged with each of the 3′ regions specified below, which contain DNA sequences complementary to and adjacent to the SNPs of interest, or they can be interchanged with other additional unique 5′ portions as specified by the micro-arrayed tags in the microplate wells provided they are used to identify blood group or HPA SNPs; and (2) the 3′ portion: the 3′ nucleotides are complementary to and precisely adjacent to the SNP site of the PCR-amplified DNA, which enables the detection of either or both nucleotides of the SNP. Thus, the extension probe is a unique sequence that can hybridized to a specific location and to the PCR-amplified DNA and be extended by a single fluorescent-labeled dideoxy-nucleotide using PCR thermal cylers. The extension probe products are hybridized to the complementary micro-arrayed DNA sequence on the microplate and the incorporation of Bodipy- and Tamra-labeled dideoxy-nucleotides are detected by laser-microplate fluorescence for each individual blood group SNP. The presence of the nucleotides for a given SNP is displayed by automated imaging and analysis software. In one variation of the detection reaction, a dideoxyguanidine tri-nucleotide labeled with the Bodipy-fluorochrome is added in the extension reaction. If a deoxycytidine is present in the

PCR-amplifed DNA fragment, then the nucleotide will be incorporated into the nascent DNA fragment. In another variation of the reaction, a dideoxyadenine nucleotide labeled with the Tamra-fluorochrome is added to the extension assay. If the PCR-amplified fragment contains a deoxythimidine, then an extension will occur. In each case, the flurochrome is detected after the extension reaction has been completed. Again, these reactions proceed in the same tube along with the other extension reactions. The laser-detection apparatus can identify and evaluate each specified extension due to the location of each micro-arrayed DNA sequence.

Each extension primer has a region complementary to a tag that is been bound to the surface of a microplate well (Bold nucleotides) and a region (Italicized nucleotides) that is complementary to the region and immediately adjacent to the SNP site.

It should be noted that the teachings, products and methods of the present invention are not limited to the above-specified primer pairs and probes, but additionally comprise all primer pairs and probes specific to the blood group and HPA SNPs, wherein said primer pairs and probes are optimized for use in a multiplex PCR reaction for the simultaneous identification of more than one, or all, blood group or HPA genotypes and their corresponding phenotypes.

Examples

Although the following examples may provide preferred methods, products, platforms or protocols of the present invention, it will be understood by one skilled in the art that the presently provided examples are not limited to the specified parameters of each example, and may be varied

provided that the resulting outcome of the methods or protocols are in accordance with the teachings of the present invention, and the products are functionally equivalent or relating to the teachings of the present invention.

Example 1

A preferred protocol for the multiplex blood group and HPA SNP Genotyping is provided. Although the present example analyzes 12 SNP extension primers, the present invention is not limited to the analysis of a maximum of 12 SNPs, but may include a plurality of SNPs relating to more than one or all of the blood group or HPA SNPs.

Additional blood group and platlet antigen SNPs for clinically relevant antigens embodied by the present invention appear in Table 1A. Primer pairs and probes, such as those exemplified in Tables 1 and 2, corresponding to these SNPs of clinical relevance, can be prepared according to the teachings of the present invention. Target primers may be initially identified from existing databases (e.g. autoprimer.com) based on information corresponding to the SNP of interest and the corresponding flanking regions, and subsequently optimized as herein disclosed for use in accordance with the present invention.

I (a). PCR Primer Pooling

Step	Action
1	Dilute each of 12 PCRS and PCRA primer (forward and reverse primers) pairs to final concentration of 240uM (only required upon arrival of new primers)
2	Generate working primer pool by combining 5 ul of each of the 24 individual PCR primers

I (b). SNP Extension Primer Pooling

Step	Action
1	Dilute each of 12 SNP extension primers to final concentration of 120uM (only required upon arrival

	of new primers)
2	Generate working SNP extension primer pool by combining 10 ul of each of the 12 individual SNP extension primers

II. Multiplex PCR from purified DNA templates

Step	Action
1	Prepare 10ul multiplex PCR master mix for use with 96 well plates containing PCR primers (synthesized by Integrated DNA Technologies, Coralville, IA, USA), dNTPs (MBI Fermentas, Hanover, MD, USA), $MgCl_2$, 10X PCR Buffer, and Amplitaq Gold (Applied Biosystems, Branchburg, NJ, USA): **Component** / **Initial Concentration** / **Final Concentration** / **Volume (ul/well)** PCR primer pool each / 0.05 / 10uM each / 50nM dNTPs / 2.5mM each / 75uM each / 0.33 $MgCl_2$ / 25mM / 5mM / 2.00 10x PCR Buffer / 10x / 1x / 1.00 AmpliTaq Gold / 5U/ul / 0.075U/ul / 0.15 dH_2O / / / 4.47
2	For each DNA Sample, transfer 2ul of 4ng/ul stock DNA to each well of 96 well plates. Use Biomek FX (Beckman Coulter Inc., Fullerton, CA, USA) Script '2ul96well Transfer' automated program
3	Place Multiplex PCR Master Mix in Biomek FX station 1. Place 96 well plates of DNA in Biomek FX station 5-8.
4	Transfer 8ul Multiplex PCR master mix to DNA samples using Biomek FX Script: '8ul PCR Transfer'
5	After addition of master mix seal tightly with MJ Microseal A film (MJ Research, Inc., Waltham, MA, USA)
6	Spin down in centrifuge for 30 sec at 1500 rpm
7	Place in MJ Tetrad Thermal cyclers (MJ Research, Inc., Waltham, MA, USA) and run 'UHT-MPX' CBS multiplex PCR program: **Thermal cycle conditions 'UHT-MPX':** Denature 94°C 1:00 (min) 35 cycles of: 94°C 0:30 (min) 55°C 0:33 (min) 72°C 1:00 (min) Hold Temperature 4°C ∞

5

III. Post PCR Cleanup

Step	Action
1	Prepare ExonucleaseI (ExoI; USB Corporation, Cleveland, OH, USA) and Shrimp Alkaline Phosphatase (SAP; USB Corporation, Cleveland, OH, USA) master mix: **Component Final concentration Volume per well (ul)** ExoI 2U 0.4 SAP 1U 2.0 10x SAP buffer 1x 0.6 dH_2O 3.0
2	Add Exo/SAP master mix to grooved reservoir and place on Multimek (Beckman Coulter Inc., Fullerton, CA, USA) Station 3
3	Add UHT (ultra high-throughput) salt solution (provided) to grooved reservoir and place on Multimek Station 4
4	Transfer 8ul Exo/SAP master mix to amplified PCR products using Multimek Script: EXO96-2.SCI (two 96 well plates, at Multimek stations 1 and 2
5	After Multimek addition of Exo/SAP seal tightly with MJ Microseal A film
6	Spin down in centrifuge for 30 sec at 1500 rpm
7	Place in MJ Tetrad Thermal cyclers and run 'UHTCLEAN' program: **Thermal cycle conditions 'UHTCLEAN':** **Temp Time (min)** 37°C 30:00 100°C 10:00 4°C ∞

IV. SNP-IT Assay using the GenomeLab SNPStream™ (Beckman Coulter Inc. Fullerton, CA, USA)

Step	Action
1	Prepare SNP-IT extension mix containing extension primers (synthesized by Integrated DNA Technologies, Coralville, IA, USA), C/T ddNTPs, Extension mix diluent, and DNA polymerase (Beckman Coulter Inc., Fullerton, CA, USA) **Component Volume per well (ul)** SNP Extension primer pool 3.22 C/T ddNTP Extension mix 21.43 Extension mix diluent 402.98 DNA polymerase 2.24 dH2O 318.22
2	Add SNP-IT mix to grooved reservoir and place on

	Multimek Station 3
3	Add UHT salt solution (provided) to grooved reservoir and place on Multimek Station 4
4	Transfer 7ul SNP-IT extension mix to UHT-CLEAN PCR products using Multimek Script: 7UL96-2.SCI (two 96 well plates, at Multimek stations 1 and 2
5	After Multimek addition of SNP-IT extension mix seal tightly with MJ Microseal A film
6	Spin down in centrifuge for 30 sec at 1500 rpm
7	Place in MJ Tetrad Thermal cyclers and run 'UHT-SNPIT' program: **Thermal cycle conditions 'UHTSNPIT':** **Temp** **Time (min)** Denature 96°C 3:00 45 cycles of: 94°C 0:20 40°C 0:11 Hold Temperature 4°C ∞

V. Post-extension Transfer and Hybridization

Step	Action
1	Preheat incubator to 42°C
2	Make sure there is adequate 20x dilution of SNPWare UHT Wash Buffer in washer Carboy B. If required dilute 20x stock solution with water and refill Carboy B
3	Run SAMI / EL 405 Script 'Prime B'
4	Place all Tag Array plates in Row 1 of the Carousel, starting with Hotel 1, with subsequent plates in Hotel 2, 3, etc., preferably with their barcodes facing inwards.
5	Place all PCR plates directly below their corresponding Tag Array Plates. PCR plates corresponding to Quadrants 1-4 should be placed in Rows 2-5 of the proper Hotel, respectively. For all PCR plates, the "ABC..." lettered edge of the plates should face inwards on the Carousel.
6	Place grooved reservoir with solubilized UHT Salt Solution in Multimek Station 4
7	Place grooved reservoir with Hybridization solution master mix in Multimek Station 3 Hybridization Solution master mix: **Component** **Volume per Tag Array plate (ul)** 2x Hybridiaztion Soluton 3500.00 Hybridization Additive 203.7

8	Run SAMI Script 'Post-extension Transfer_Hybridization 1x384.smt': This automated program prepares the tag array plate by washing it 3x with SNPWare UHT wash buffer; adds 8.0ul of Hybridization solution master mix to each SNP extension reaction and subsequently transfers 8.0ul of this mixture to the prepared tag array plate.
9	Place Tag Array plates in humidified 42°C incubator for 2 hours

VI. Post-Hybridization Wash

Step	Action
1	Make sure there is adequate 64x dilution of SNPWare UHT Stringent Wash Solution in washer Carboy C. If required dilute 64x stock solution with water and refill Carboy C
2	Run SAMI / EL 405 Script 'Prime C'
3	Run SAMI / EL 405 Script 'Post-hyb 3x Wash'
4	Completely dry Tag Array plates using vacuum/pipette tip
5	Run SAMI / EL405 script 'Prime A' several times to clean plate washer pins

VII. UHT (Ultra high through-put) Tag Array Plate Reading

Step	Action
1	Turn on lasers, turning both keys 90 degrees clockwise, and allow at least 30 minutes to warm up
2	Turn on SNPScope Reader and Twister.
3	Activate lasers: Flip two switches on laser box from 'Standby' to 'Operate'/'Laser'
4	Open UHT Run Manager Software and 'Initialize' SNPScope system
5	Stack Tag Array plates in Twister carousel 1, with 'Assay Test Plate' on top. Make sure all barcodes are facing outwards, and plates are pushed towards the reader
6	Select 'SNPTEST_W_BC_run' from UHT RUN Manager Software, enter the number of plates to be read (including the test plate).
7	Select 'RUN'

The SNPScope plate reader will excite and capture images of Bodipy-fluorescein and Tamra- labeled ddNTPs separately. All genotype calls are subsequently

automatically generated using the SNPStream Software Suite of MegaImage, UHTGetGenos and QCReview.

It should be noted that the specific steps associated with the protocol exemplified in Example 1 are not intended to limit the teachings and methods of the present invention to the specific above protocol. Example 1 is provided to specify a preferred method in accordance with the present invention wherein a plurality of blood group and HPA SNPs are simultaneously analysed in a ultra high throughput multiplex automated system for the determination of the specific genotypes and accordingly the phenotypes associated therewith. Accordingly, it should be understood by one skilled in the art that the steps of Example 1 may be varied provided that such variations yield the preferred results of the present invention.

RESULTS

1. GP3A Exon 3 SNP Scatter Plots.

The robotic UHT platform produces laser-fluorescence values for each sample which are represented in 'scatter plots' for the operator to review. A sample scatter plot is shown in Fig. 1 for the SNP analysis GP3A Exon 3, which represents the HPA-1a and HPA-1b antigens. As can been seen in Fig. 1 and Fig. 4, results are graphed using logarithmic and XY scatter plots (upper right). Green, orange or blue sample designations represent CC, TC and TT SNP genotype calls, respectively. No fluorescence represents an assay failure (FL) for that sample.

Scatter plots (as shown in Fig. 1 and Fig. 4) are generated preferably using SNPStream software suite and viewed through QCReview. It should be additionally noted that the present analysis is not limited to SNPstream or

QCReview, and may be carried out using any SNP analysis software. Individual TT, TC and CC genotype calls are represented as dark blue, orange and green open circles, respectively. Sample failures and water controls are represented by yellow and light blue filled circles respectively. Logarithmic (left) and XY scatter (upper right) plots are generated using the relative fluorescence of the Bodipy-fluorescein and Tamra labels obtained during SNPScope plate imaging and analysis.

2. SNP Data Manipulation and Analysis.

The SNP results of a scatter plot are electronically exported to a spreadsheet and examined for total sample failure and individual SNP failure rates. SNP results for 372 DNA samples are summarized in Table 3 (provided in Appendix A). Accordingly, Table 3 provides the Pass and Failure Rates for 12 blood group and HPA SNP analyses. 372 DNA samples were analyzed for several antigens, including the blood group RhD (RHD Exon 4 and RHD Exon 9) and platelet HPA-1a/b (GP3A Exon 3) genotypes. Sample success or pass rates are indicated on the right and individual SNP success or pass rates are shown at the bottom. Three hundred and fifty seven of 372 samples (96%) had results for at least one SNP. Individual SNP results (i.e. minus the sample failures) ranged from 80-100%; only two SNPs had success rates <98%. Individual SNP failures do not affect the results of a sample for other SNPs that do not fail.

3. SNP Allele Result Compared to the Serological Result

RhD status was compared between the serological result and the SNP analysis for RHD Exon 4 and RHD Exon 9. Table 4 summarizes the comparison. 287 of 291 (98.6%) RhD positive units and 55 of 66 (83.3%) RhD negative units were

identified correctly using the UHT SNP platform. It is important to note that the 6 incorrect calls suggesting the presence of the RHD gene in a serologically RhD-negative sample may be due to one of the non-functional RHD genes present in the random population (Singleton B.K. et al., Blood 2000;95:12; Okuda H., et al., J Clin Invest 1997;100:373; Wagner F.F. et al., BMC Genet 2001;2:10).

Table 4. A comparison of the SNP genotype result and the serological result obtained with government-regulated antisera.

D-positive:
N = 291

Assay	RHD Exon 4	RHD Exon 9	No	Percent
	pos	Pos	287	98.6%
	neg	Neg	4	1.4%
Total			291	

D-negative:
N = 66

Assay	RHD4	RHD9	No	Percent
	neg	Neg	55	83.3%
	neg	FL	5	7.6%
	pos	Pos	6	9.1%
Total			66	

NOTE: CBS laboratory regulations do not allow copies of serological results of blood donors to be made from their laboratory information system. Therefore, the results of the CBS serological phenotypes were reviewed by research personnel and the results tabulated and compared to the SNP data.

4. SNP Genotype Frequency Analysis.

The SNP results then were compared with published phenotype frequencies for Caucasians and Blacks and are summarized in Table 5 below. The data clearly shows that the allele frequencies are consistent with the accepted published frequencies for Caucasians and Blacks. The data show that the SNP genotype frequencies match the published population phenotype frequencies.

Table 5. A summary of the UHT SNP analysis of genotype frequencies for several SNPs analyzed and compared to published phenotype frequencies for Caucasians and Blacks. The ethnicity of the samples analyzed is not known.

Table 5

UHT Genotyping Analysis

FL = assay failure

KEL Exon6

Phenotype	Caucasians	Blacks	Observed	(%)
K-k+	91%	98%	326	91.3
K+k-	0.2%	rare	0	0
K+k+	8.8%	2%	28	7.8
Fails			3	0.8
No of FL	18			
No.of Pass	354			
Call Rate	**95.2%**			

An independent assay as described in Molecular Protocols in Transfusion Medicine was performed using the UHT SNP Stream System.
Seven samples were tested (Four KEL 2/KEL 2, Three KEL1/KEL 2).
All samples showed a 100% correspondence with the UHT genotype results.

KEL Exon8

Phenotype	Caucasians	Blacks	Observed	(%)
Kp(a+b-)	Rare	0%	0	0
Kp(a-b+)	97.7%	100%	354	99.2
Kp(a+b+)	2.3%	rare	1	0.3
Fails			2	0.6
No of FL	17			
No.of Pass	355			
Call Rate	**95.4%**			

DI Exon18

Phenotype	Caucasians	Blacks	Observed	(%)
Di(a+b-)	<0.01%	<0.01%	0	0
Di(a-b+)	>99.9%	>99.9%	353	98.9
Di(a+b+)	<0.1%	<0.1%	2	0.6
Fails			2	0.6
No of FL	17			
No.of Pass	355			
Call Rate	**95.4%**			

FY PRM

Phenotype	Observed	(%)
wt/wt	348	97.5
wt/mut	7	20
mut/mut	2	0.5
Fails	0	0
No of FL	15	
No.of Pass	357	
Call Rate	**96.0%**	

An independent assay as described in Molecular Protocols in Transfusion Medicine was performed using the UHT SNP Stream System.
Thirteen samples were tested (six wt/wt, five wt/mut and two mut/mut for the GATA site).
All samples showed a 100% correspondence with the UHT genotype results.

FY Exon 2

Phenotype	Caucasians	Blacks	Observed	(%)
Fy(a+b-)	17%	9%	89	24.9
Fy(a-b+)	34%	22%	112	31.4
Fy(a+b+)	49%	1%	155	43.4
Fails			1	0.3
No of FL	16			
No.of Pass	356			
Call Rate	**95.7%**			

An independent assay as described in Molecular Protocols in Transfusion Medicine was performed using the UHT SNP Stream System
Eleven samples were tested (eight FY2/FY2, three FY1/FY2 and one FY1/FY1).
All samples showed a 100% correspondence with the UHT genotype results.

GP3A Exon 3

Phenotype	Caucasians	Blacks	Observed	(%)
HPA-1a/1a	80%	84%	263	73.7
HPA-1a/1b	18%	64%	89	24.9
HPA-1b/1b	2%	0%	4	1.1
Fails			1	0.3
No of FL	16			
No.of Pass	356			
Call Rate	**95.7%**			

An independent assay as described in Molecular Protocols in Transfusion Medicine was performed *using the UHT SNP Stream System.*
Eighteen samples were tested (Seven HPA-1a, Seven HPA-1a/1b and Four HPA-1b).
All samples showed a 100% correspondence with the UHT genotype results.

JK9

Phenotype	Caucasians	Blacks	Observed	(%)
Jk(a+b-)	26.3%	51.1%	90	25.2
Jk(a-b+)	23.4%	8.1%	87	24.4
Jk(a+b+)	50.3%	40.8%	178	49.4
Fails			2	0.5

No of FL	17
No.of Pass	355
Call Rate	**95.4%**

An independent assay as described in Molecular Protocols in Transfusion Medicine was performed using the UHT SNP Stream System .
Nineteen samples were tested (Seven JK1, Seven JK1/JK2 and Five JK2).
All samples showed a 100% correspondence with the UHT genotype results.

5. HPA-1a/HPA-1b PCR-RFLP Analysis.

The GP3A Exon 3 SNP detection method for HPA-1a/b genotyping (Appendix A) was compared to a subset of samples (n = 18) using conventional PCR-RFLP analysis performed independently (Fig. 2). The results of the two assays were 100% concordant. In addition, a 217G nucleotide mutation 21 basepairs downstream of the GP3A SNP was present in sample 8. This mutation does not affect HPA-1b expression but is detected in the PCR-RFLP and is prone to interpretation error in the conventional PCR-RFLP assay. However, the sample was correctly genotyped as HPA-1b in our SNP assay. Accordingly, the present invention eliminates or minimizes error in HPA-1 results obtained since no confusing or confounding information results from the gel readings of the present invention. That is to say, the conventional RFLP detected the presence of an additional DNA fragment at ~180bp which represents a heterozygous HPA-1b/1b^{G217} allele and was correctly genotyped as HPA-1b/b by the present invention.

Example 2

However, it should be obvious to one skilled in the art that other methodologies and/or technologies for SNP

identification could be used, providing that the novel DNA sequences disclosed above are also used. Other embodiments could include the following but without limitation to micro-arrays on glass slides or silica chips, the use of mass spectrometry, or oligo-ligation and extension techniques to detect the SNPs of interest.

A preferred method of the present invention relates to a method for the detection of blood group and HPA genotypes. The present invention also provides novel DNA sequences that are used as primers in a multiplex PCR format according to the present invention to amplify the genomic regions of interest. The present invention also provides novel combinations of DNA sequences that are used in said multiplex PCR format, and for novel DNA sequences that are used to detect blood group and platelet SNPs.

A preferred application of the present invention is in the blood collection and blood banking industry without limitation to red blood cell, platelet, and bone marrow donations. Canada has over 850,000 blood donations yearly, many from repeat donors. Eventually, after all repeat donors are tested (each donor is tested once), the analyses will be performed only on the blood of new donors. With over 29 blood group and 6 HPA systems encompassing over 250 antigens, the platform will find wide application in this industry.

The present invention additionally encompasses various embodiments relating to the detection of various SNPs for the determination of the various genotypes in a sample and for the determination of the corresponding phenotype. In a preferred embodiment, the present invention utilizes a platform to analyzes a cytidine-to-thymidine (C→T) single nucleotide polymorphism. The invention may also employ the

multiplex detection of, but not limited to, C→A, A→T, and G→C SNPs, or any other nucleotide SNP related to blood group or platelet antigens.

The present invention may additionally include methods and products for the detection of clinically relevant blood group antigens whereby an antigen of interest is characterized by a genotypic identifier that exceeds a single nucleotide polymorphism. Specifically, the present invention may extend to include clinically relevant insertions or deletions or other nucleotide changes that characterize a blood group antigen of interest, such a multiple base pair insertion in an allele of interest. For example, a genotypic identifier corresponding to a blood group antigen of interest may be pre-characterized, suitable primers and probes for detection thereof may be prepared and a blood sample screened according to the teachings of the present invention.

The present invention provides DNA sequences corresponding to the PCR primer pairs optimized for multiplex use to identify blood group and platelet antigens simultaneously. Accordingly, the present invention provides the novel primer pair sequences listed in Table 1.

The present invention additionally provides novel DNA sequences used to identify the single nucleotide polymorphisms (SNPs) that represent underlying DNA blood group and platelet antigens. Accordingly, the present invention provides the novel extension probes listed in Table 2.

The present invention provides a method of a combined analysis of blood group and HPA SNP analyses.

The present invention advantageously utilizes PCR, the variant and unique SNPs for the variant alleles that infer blood group phenotypes, and single base extension and detection chemistry as a foundation for the novel products and methods of the present invention. Accordingly, the present invention provides a high throughput, multiplexed, DNA-based method of blood group genotyping that replaces the current manual, semi-automated and automated serological screening process used to determine blood group phenotypes.

Accordingly, the present invention provides a method for the identification of rare blood group genotypes due to the suite of SNPs as described above, and in some instances replaces the current state of the art in which most rare blood group genotypes are identified serendipitously (propositus and their relatives) and enabling significant advances over current serological technologies. For example, by analyzing the SNP for the RhC allele in Rh negative blood, we can identify RhC homozygotes and thereby, the rare RhD-negative and Rhc-negative blood.

The present invention additionally provides a method of use in tissue compatibility matching for the purposes, without limitation, of organ transplantation, bone marrow transplantation and blood transfusion related to blood group and platelet antigens.

The present invention additionally provides novel components and constituents that are beneficial for the analyses relating to the present invention. More specifically, the group of currently developed SNPs representing a 'suite', or the presently known set of SNPs that relate to clinically important blood group and HPA genotypes for red blood cell and platelet antigens,

respectively are provided. The present invention is not limited to the presently listed SNPs, but is understood to comprise all blood group and platelet antigen, and preferably HPA SNPs that may be analyzed in accordance with the teachings of the present invention and using the products, protocols and methods of the present invention.

The present invention also provides the DNA primer sequences optimized for use in a multiplex PCR format.

The present invention also provides novel DNA probe sequences used to detect the SNPs of interest.

The present invention provides a method for the simultaneous detection of a plurality of blood group SNPs. More specifically, the present invention provides a method for the simultaneous detection of at least 19 blood group SNPs; *RHD (2), RHC/c, RHE/e, S/s, Duffy (a/b), Kidd (a/b), Diego (a/b), Kell K1/K2, Kell K3/K4, and HPA-1a/b* simultaneously. The method of the present invention provides (1) DNA sequences corresponding to the PCR primer pairs optimized for multiplex use to identify a plurality of blood group and platelet antigens simultaneously; (2) Novel DNA sequences used to identify the single nucleotide polymorphisms (SNPs) that represent underlying DNA blood group and platelet antigens; and (3) The combination of SNP analyses including blood group and platelet antigens.

To support and validate the teachings of the present invention various experimental tests have been completed and analyzed. Numerous validating experimental data has been recorded, however, for the purpose of simplicity the following example is provided. Each step in the validating experiment is noted below:

(1) Ultra high throughput (UHT) Multiplex SNP analyses on 372 unrelated blood donor specimens for RHD (2), RHC/c, RHE/e, S/s, FY1/FY2 (2), JK1/JK2, DI1/DI2, KEL1/KEL2, KEL3/KEL4, and HPA-1A/B genotypes and corresponding phenotypes was examined, and data was recorded (please refer to Appendix A for the raw data accumulated, and Table 5 for a Summary of the results obtained).

(2) Manual PCR-RFLP analyses was performed on some of the 372 specimens for some of the blood group SNPs to for comparison to the results obtained in Step (1).

(3) Serological analyses was also performed on some of the 372 specimens for each of the blood group and HPA SNPs using Health Canada regulated reagents performed by licensed medical technologists in a provincially licensed laboratory.

(4) Serological analyses was also performed on some of the 372 specimens for each of the blood group and platelet antigens by unlicensed research technologists using Health Canada regulated reagents and methodologies in an unlicensed laboratory.

The results obtained from the above validating experimental data is provided below by way of supportive Figures and Tables.

1. SNP Platform data generation.

The robotic platform produces fluorescence for each sample which are presented in 'scatter plots' (as illustrated in Fig. 1) for the operator to review. Sample genotype results are shown for each blood group SNP and are graphed using logarithmic and XY scatter plots (upper right). Green, orange or blue sample designations represent

CC, TC and TT genotype calls respectively. No fluorescence represents an assay failure (FL) for that sample.

2. SNP Data Manipulation and Analysis.

The SNP results of a scatter plot are electronically exported to a spreadsheet and examined for total sample failure and individual SNP failure rates. Twelve SNP results for 372 DNA samples are summarized in Table 3 with sample failure rates (shown on the right) and individual SNP success rates (shown at the bottom). Three hundred and fifty seven of 372 samples (96%) had results for at least one SNP. Individual SNP results ranged from 80% to 100%; only one SNP result success rate was <98%. Individual SNP failures do not affect the results of a sample for other SNPs that do not fail.

3. SNP Allele Frequency Analysis.

The SNP results where then compared with published phenotype frequencies for Caucasians and Blacks and are summarized in Table 5 above. The data shows that the allele frequencies are consistent with the accepted published frequencies for Caucasians and Blacks.

3.1 SNP Allele Result Compared to the Serological Result.

RhD status was compared between the serological result and the SNP analysis for RHD exon 4, and 9 (RHD Exon 4, RHD Exon 9, respectively). Table 4 summarizes the comparison. 287 of 291 (98.6%) RhD positive units and 55 of 66 (83.3%) RhD negative units were identified correctly using the UHT SNP platform.

3.2 SNP Analysis compared to Manual PCR-RFLP.

Some of the UHT SNP genotype results were compared with manual PCR-RFLP analysis performed independently. The results show 100% concordance. A representative PCR-RFLP is shown in Fig. 3.

The genotyping technology provided in the present invention queries and analyzes SNPs using single base-pair primer extension. In brief, the genomic region surrounding the SNP of interest is amplified and used as a template for the ensuing hybridization and single nucleotide extension of the SNP specific extension primer. The extension primer is designed to hybridize adjacent to the polymorphic nucleotide(s) and enables us to query bi-allelic polymorphisms, small insertions, deletions or inversions. The 5′ extension primer tags are hybridized to the complementary DNA sequence on micro-arrayed plates and incorporation of Bidopy- and Tamra-labeled ddNTPs are detected by laser-microplate fluorescence for each individual blood group and HPA SNP. Individual sample genotypes are generated through automated imaging and analysis software as shown in the genotype scatter plots of Fig. 1.

The embodiment(s) of the invention described above is(are) intended to be exemplary only. The scope of the invention is therefore intended to be limited solely by the scope of the appended claims.

Appendix A Genotype Results for updated 12 SNP CBS Panel

Sample ID	RHD4	RHD7	RHD9	RHCE2	RHCE5
BB24401	FL	FL	FL	FL	FL
BB24402	TT	FL	CC	CC	TC
BB24407	TC	TT	TC	TC	TC
BB24408	TC	TT	TC	TC	CC
BB24409	TC	TT	TC	TC	TC
BB24410	TC	TT	TC	TC	TC
BB24415	TC	TT	TC	TC	FL
BB24416	FL	FL	FL	FL	FL
BB24417	TC	TT	TC	FL	TC
BB24420	TC	TT	TC	TC	CC
BB24421	TC	TT	TC	TC	CC
BB24422	TC	TT	TC	FL	TC
BB24423	TC	TT	TC	TC	TC
BB24424	TC	TT	TC	FL	TC
BB24425	TC	TT	TC	TC	CC
BB24426	TC	TT	TC	TC	TC
BB24427	TC	TT	TC	TC	TC
BB24428	TC	TT	TC	TC	CC
BB24429	TC	TT	TC	TC	CC
BB24430	TC	TT	TC	TC	TC
BB24431	TC	TT	TC	TC	CC
BB24432	TC	TT	TC	TC	CC
BB24433	TC	TT	TC	TC	CC
BB24434	TC	TT	TC	TC	CC
BB24435	TC	TT	TC	TC	CC
BB24436	TT	FL	CC	CC	TC
BB24437	TC	TT	TC	TC	TC
BB24438	TC	TT	TC	TC	TC
BB24439	TC	TT	TC	TC	CC
BB24440	TC	TT	TC	FL	TC
BB24444	TC	TT	TC	TC	CC
BB24448	TT	FL	CC	CC	FL
BB24461	TC	TT	TC	TC	CC
BB24462	TT	FL	CC	CC	TC
BB24463	TC	TT	TC	TC	CC
BB24464	TC	TT	TC	TC	CC
BB24465	TC	TT	TC	TC	TC
BB24466	TC	TT	TC	FL	TC
BB24467	TT	FL	CC	CC	TC
BB24468	TC	TT	TC	FL	TC
BB24469	TC	TT	TC	TC	CC
BB24470	TT	FL	CC	CC	TC
BB24471	TC	TT	TC	TC	TC
BB24472	TC	TT	TC	TC	TC
BB24473	TC	TT	TC	TC	TC
BB24474	TC	TT	TC	TC	TC
BB24475	TC	TT	TC	TC	TC
BB24476	TC	TT	TC	TC	CC
BB24477	TC	TT	TC	TC	TC
BB24478	TC	TT	TC	TC	TC
BB24479	TC	TT	TC	TC	TC
BB24480	TC	TT	TC	TC	TC

BB24481	TC	TT	TC	TC	CC
BB24482	TC	TT	TC	TC	TC
BB24483	TT	FL	CC	CC	TC
BB24484	TC	TT	TC	TC	TC
BB24485	TT	FL	CC	FL	TC
BB24486	TT	FL	CC	CC	TC
BB24487	TC	TT	TC	TC	TC
BB24488	TC	TT	TC	TC	TC
BB24489	TC	TT	TC	TC	TC
BB24491	TC	TT	TC	TC	TC
BB24492	TT	FL	CC	CC	TC
BB24493	TT	FL	CC	CC	TC
BB24494	FL	FL	FL	FL	FL
BB24495	TC	TT	TC	TC	TC
BB24496	TC	TT	TC	TC	TC
BB24497	TC	TT	TC	TC	CC
BB24499	TC	TT	TC	TC	TC
BB24504	TC	TT	TC	TC	TC
BB24505	TC	TT	TC	TC	TC
BB24506	TC	TT	TC	TC	CC
BB24507	TC	TT	TC	TC	CC
BB24512	TT	FL	CC	CC	TC
BB24513	TC	TT	TC	FL	TC
BB24516	TC	TT	TC	TC	CC
BB24517	TT	FL	CC	CC	TC
BB24518	TC	TT	TC	TC	TC
BB24519	TC	TT	TC	TC	CC
BB24522	TC	TT	TC	TC	CC
BB24523	FL	FL	FL	FL	FL
BB24524	TC	TT	TC	TC	CC
BB24525	TC	TT	TC	FL	TC
BB24526	TC	TT	TC	TC	CC
BB24527	TT	FL	CC	CC	TC
BB24528	TC	TT	TC	TC	TC
BB24529	TC	TT	TC	TC	TC
BB24530	TC	TT	TC	TC	CC
BB24531	FL	FL	FL	FL	FL
BB24532	TC	TT	TC	TC	CC
BB24533	TC	TT	TC	TC	TC
BB24534	TC	TT	TC	FL	TC
BB24535	TC	TT	TC	TC	TC
BB24536	TC	TT	TC	TC	TC
BB24537	TC	TT	TC	TC	CC
BB24538	TC	TT	TC	TC	CC
BB24539	TC	TT	TC	TC	CC
BB24540	TC	TT	TC	TC	TC
BB24541	TC	TT	TC	FL	TC
BB24542	TC	TT	TC	TC	CC
BB24543	TC	TT	TC	TC	CC
BB24547	FL	FL	FL	FL	FL
BB24548	TT	FL	CC	CC	TC
BB24549	TT	FL	CC	CC	FL
BB24550	TC	TT	TC	TC	TC
BB24552	TC	TT	TC	TC	TC
BB24553	TC	TT	TC	TC	CC
BB24554	TC	TT	TC	FL	TC

BB24555	TC	TT	TC	TC	TC
BB24556	TC	TT	TC	TC	CC
BB24557	TC	TT	TC	TC	CC
BB24558	TC	TT	TC	TC	TC
BB24559	TC	TT	TC	TC	TC
BB24560	TT	FL	CC	CC	CC
BB24561	TC	TT	TC	FL	TC
BB24562	TC	TT	TC	TC	CC
BB24563	TT	FL	CC	CC	TC
BB24564	TC	TT	TC	TC	CC
BB24565	TC	TT	TC	TC	TC
BB24566	TT	FL	CC	CC	TC
BB24567	TC	TT	TC	TC	CC
BB24568	TC	TT	TC	TC	CC
BB24569	TC	TT	TC	TC	TC
BB24570	TC	TT	TC	FL	TC
BB24571	TC	TT	TC	TC	TC
BB24572	TT	FL	CC	CC	TC
BB24573	TC	TT	TC	TC	TC
BB24574	TT	FL	CC	CC	TC
BB24575	TT	FL	CC	CC	TC
BB24576	TC	TT	TC	TC	TC
BB24577	TC	TT	TC	TC	TC
BB24578	TC	TT	TC	TC	TC
BB24579	TC	TT	TC	TC	TC
BB24580	TT	FL	FL	CC	TC
BB24581	TC	TT	TC	TC	TC
BB24586	TC	TT	TC	TC	TC
BB24587	TC	TT	TC	TC	CC
BB24594	TC	TT	TC	TC	TC
BB24600	TC	TT	TC	FL	TC
BB24601	TC	TT	TC	FL	TC
BB24602	TC	TT	TC	TC	CC
BB24603	TC	TT	TC	FL	TC
BB24604	TC	TT	TC	TC	TC
BB24605	TC	TT	TC	TC	TC
BB24606	TC	TT	TC	TC	CC
BB24607	TC	TT	TC	TC	TC
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BB24614	TC	TT	TC	TC	CC
BB24615	TC	TT	TC	TC	CC
BB24616	TC	TT	TC	TC	TC
BB24617	TC	TT	TC	FL	TC
BB24618	TC	TT	TC	TC	TC
BB24619	TC	TT	TC	FL	FL
BB24620	TC	TT	TC	FL	FL
BB24621	TC	TT	TC	TC	TC
BB24622	TC	TT	TC	FL	TC
BB24623	TT	FL	CC	CC	TC
BB24624	TT	FL	CC	CC	TC
BB24625	TC	TT	TC	TC	TC

BB24626	TC	TT	TC	TC	CC
BB24627	TC	TT	TC	TC	TC
BB24628	TC	TT	TC	TC	TC
BB24629	FL	FL	FL	FL	FL
BB24630	TC	TT	TC	TC	CC
BB24631	TC	TT	TC	FL	TC
BB24632	TC	TT	TC	TC	TC
BB24633	TC	TT	TC	FL	TC
BB24634	TC	TT	TC	TC	TC
BB24635	TC	TT	TC	TC	TC
BB24636	TC	TT	TC	FL	TC
BB24637	TC	TT	TC	FL	TC
BB24638	TC	TT	TC	TC	TC
BB24639	TT	FL	CC	CC	TC
BB24640	TC	TT	TC	TC	CC
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BB24642	TC	TT	TC	TC	CC
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BB24674	TC	TT	TC	TC	CC
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BB24676	TC	TT	TC	FL	TC
BB24678	TT	FL	CC	CC	TC
BB24679	TC	FL	TC	TC	TC
BB24680	TC	TT	TC	TC	CC
BB24681	TC	TT	TC	TC	TC
BB24682	TC	TT	TC	TC	TC
BB24683	TT	FL	CC	CC	TC

BB24684	TC	TT	TC	TC	CC
BB24685	TC	TT	TC	TC	CC
BB24686	TC	TT	TC	TC	CC
BB24687	TC	TT	TC	TC	TC
BB24688	TT	FL	CC	CC	TC
BB24689	TC	TT	TC	TC	CC
BB24690	TC	TT	TC	TC	CC
BB24691	TC	TT	TC	TC	CC
BB24692	TC	TT	TC	TC	CC
BB24693	TC	TT	TC	TC	TC
BB24694	TT	FL	CC	CC	TC
BB24695	TT	FL	CC	CC	TC
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BB24698	TC	TT	TC	TC	CC
BB24699	TC	TT	TC	TC	TC
BB24700	FL	FL	FL	FL	FL
BB24701	TT	FL	CC	CC	TC
BB24702	TT	FL	CC	CC	TC
BB24703	TT	FL	CC	CC	TC
BB24704	TC	TT	TC	FL	TC
BB24705	TC	TT	TC	TC	TC
BB24706	TT	FL	FL	CC	TC
BB24707	TC	TT	TC	TC	CC
BB24708	TC	TT	TC	FL	TC
BB24709	TC	TT	TC	TC	TC
BB24710	TC	TT	TC	TC	CC
BB24711	TC	FL	TC	TC	TC
BB24712	TC	TT	TC	TC	TC
BB24713	TC	FL	TC	FL	TC
BB24714	TT	FL	CC	CC	TC
BB24715	TT	FL	CC	CC	TC
BB24716	TC	TT	TC	TC	CC
BB24717	TC	TT	TC	TC	TC
BB24718	TC	TT	TC	TC	TC
BB24719	TC	TT	TC	TC	TC
BB24720	TC	TT	TC	TC	CC
BB24721	TC	TT	TC	TC	TC
BB24722	TC	TT	TC	TC	TC
BB24723	TC	TT	TC	TC	CC
BB24724	TC	TT	TC	TC	TC
BB24725	TT	FL	CC	CC	TC
BB24726	TC	TT	TC	TC	TC
BB24727	TC	TT	TC	TC	CC
BB24728	TC	TT	TC	TC	TC
BB24729	TC	TT	TC	FL	TC
BB24730	TC	FL	TC	TC	CC
BB24731	TC	TT	TC	TC	CC
BB24732	TT	FL	CC	CC	TC
BB24733	TC	TT	TC	TC	TC
BB24734	TC	TT	TC	TC	CC
BB24735	TT	FL	CC	CC	TC
BB24736	TC	TT	TC	TC	TC
BB24737	TC	TT	TC	FL	TC
BB24738	TT	FL	CC	CC	TC
BB24739	TT	FL	CC	CC	TC

BB24740	FL	FL	FL	FL	FL
BB24741	TC	TT	TC	TC	TC
BB24742	TC	TT	TC	TC	TC
BB24743	TC	TT	TC	TC	TC
BB24744	TC	TT	TC	TC	TC
BB24745	TC	TT	TC	TC	TC
BB24746	TC	TT	TC	TC	TC
BB24747	TC	TT	TC	TC	CC
BB24748	TC	TT	TC	TC	TC
BB24749	TT	FL	CC	CC	CC
BB24750	TC	TT	TC	TC	TC
BB24751	TC	TT	TC	TC	TC
BB24752	TC	TT	TC	TC	TC
BB24753	FL	FL	FL	FL	FL
BB24754	TC	TT	TC	TC	CC
BB24755	TT	FL	CC	CC	TC
BB24756	TC	FL	TC	TC	CC
BB24757	TC	TT	TC	FL	TC
BB24758	TC	TT	TC	TC	TC
BB24759	TC	TT	TC	TC	TC
BB24760	TC	TT	TC	TC	TC
BB24761	TC	TT	TC	TC	CC
BB24762	TC	TT	TC	TC	TC
BB24763	TC	TT	TC	TC	FL
BB24764	TT	FL	CC	TC	TC
BB24765	TC	FL	TC	TC	CC
BB24766	TC	TT	TC	FL	TC
BB24767	TC	TT	TC	TC	TC
BB24768	TC	TT	TC	FL	TC
BB24769	TC	TT	TC	TC	CC
BB24770	TT	FL	CC	CC	CC
BB24771	TC	TT	TC	TC	TC
BB24772	TC	TT	TC	TC	TC
BB24773	TC	TT	TC	TC	TC
BB24774	TC	TT	TC	TC	CC
BB24775	TC	TT	TC	TC	TC
BB24776	TC	TT	TC	TC	TC
BB24777	TC	TT	TC	TC	TC
BB24778	TC	TT	TC	TC	CC
BB24779	TC	FL	TC	TC	CC
BB24780	TC	TT	TC	TC	TC
BB24781	TC	TT	TC	TC	TC
BB24782	TC	TT	TC	TC	TC
BB24783	TT	FL	CC	CC	TC
BB24784	TC	TT	TC	TC	TC
BB24785	TC	TT	TC	TC	TC
BB24786	TC	TT	TC	TC	TC
BB24787	TC	TT	TC	TC	TC
BB24788	TC	TT	TC	TC	CC
BB24789	TC	TT	TC	TC	TC
BB24790	TT	FL	CC	CC	TC
BB24791	TT	FL	CC	FL	TC
BB24792	TC	TT	TC	TC	CC
BB24793	TT	FL	CC	CC	FL
BB24794	TC	TT	TC	TC	CC
BB24795	TC	TT	TC	TC	TC

BB24796	TC	TT	TC	TC	TC
BB24797	TC	TT	TC	TC	TC
BB24798	TC	TT	TC	TC	TC
BB24799	TC	TT	TC	TC	TC
BB24800	TC	TT	TC	TC	TC
BB24801	FL	FL	FL	FL	FL
BB24803	TC	TT	TC	TC	CC
BB24804	TT	FL	CC	CC	TC
BB24805	TC	TT	TC	TC	CC
BB24806	TC	TT	TC	TC	CC
BB24807	TC	TT	TC	FL	TC
BB24808	TC	TT	TC	TC	TC
BB24809	TC	TT	TC	TC	TC
BB24810	TC	TT	TC	TC	CC
BB24811	FL	FL	FL	FL	FL
BB24812	TC	TT	TC	TC	TC
BB24815	TC	TT	TC	TC	TC
BB24817	TC	TT	TC	TC	TC
BB24818	TC	TT	TC	TC	TC
BB24819	TC	TT	TC	TC	CC
BB24820	TC	TT	TC	TC	TC
BB24821	TT	FL	CC	CC	TC
BB24823	TC	TT	TC	TC	TC
BB24824	TC	TT	TC	TC	TC
BB24826	TC	TT	TC	TC	TC
BB24827	TT	FL	CC	TC	TC
BB24830	TC	TT	TC	TC	TC
BB24831	TC	TT	TC	TC	CC
BB24832	TT	FL	CC	CC	TC
BB24833	TC	TT	TC	TC	TC
BB24834	TC	TT	TC	TC	CC
BB24836	TC	TT	TC	TC	TC
BB24837	TC	TT	TC	TC	TC
BB24838	TC	TT	TC	TC	TC
BB24839	TC	TT	TC	TC	CC
BB24841	TC	TT	TC	TC	TC
BB24842	TC	TT	TC	TC	TC
BB24843	TT	FL	FL	TC	FL
BB24844	FL	FL	FL	FL	FL
BB24847	TC	TT	TC	TC	CC
Q1H2O	FL	FL	FL	FL	FL
Q2H2O	FL	FL	FL	FL	FL
Q3H2O	FL	FL	FL	FL	FL
Q4H2O	FL	FL	FL	FL	FL

	RHD4	RHD7	RHD9	RHCE2	RHCE5
Sample FL	15	86	20	54	23
Sample Pass	357	286	352	318	349
Call Rate	95.97%	76.88%	94.62%	85.48%	93.82%
Genotypes (N)					
XX (TT)	64	286	0	0	0
XY (TC)	293	0	293	260	246
YY (CC)	0	0	59	58	103
Allele Freq					
X (p)	58.96%	100.00%	41.62%	40.88%	35.24%
Y (q)	41.04%	0.00%	58.38%	59.12%	64.76%

KEL6	KEL8	DI18	FYP	FY2	GP3A
FL	FL	FL	FL	FL	FL
CC	CC	CC	TT	CC	TC
CC	CC	CC	TT	TT	TC
CC	CC	CC	TT	CC	TT
CC	CC	CC	TT	CC	CC
CC	CC	CC	TT	TC	TT
CC	CC	CC	TT	TT	TT
FL	FL	FL	FL	FL	FL
CC	CC	CC	TT	CC	TT
CC	CC	CC	TT	CC	TT
CC	CC	CC	TT	TC	TT
CC	CC	CC	TT	TC	TC
TC	CC	CC	TT	TC	TT
CC	CC	CC	TT	TT	TT
CC	CC	CC	TT	TC	TT
TC	CC	CC	TT	TT	TC
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CC	CC	CC	TT	TC	TT

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TC	CC	CC	TT	CC	TC
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CC	CC	CC	TT	TT	TT
CC	CC	CC	TT	CC	TT
CC	CC	CC	TT	TC	TT
FL	FL	FL	FL	FL	FL
CC	CC	CC	TT	TC	TC
CC	CC	CC	TT	TT	TT
CC	CC	CC	TT	TC	TC
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CC	CC	CC	TT	TC	TT
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CC	CC	CC	TT	TT	TT

CC	CC	CC	TT	TC	TC
CC	CC	CC	TT	CC	TT
TC	CC	CC	TT	TC	TC
FL	FL	FL	FL	FL	FL
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CC	CC	CC	TT	TT	TC
FL	FL	FL	FL	FL	FL
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CC	CC	CC	TT	TC	TT
FL	CC	FL	TT	TC	CC
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CC	CC	CC	TT	TC	TC
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CC	CC	CC	TT	TT	TT
TC	CC	CC	TT	TT	TT
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CC	CC	CC	TT	TC	TT
FL	FL	FL	FL	FL	FL
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CC	CC	CC	TT	TT	TT
CC	CC	CC	TT	TC	TC
CC	CC	CC	TT	TC	TC

FL	FL	FL	FL	FL	FL
CC	CC	CC	TT	TC	TT
CC	CC	CC	TC	TT	TT
CC	CC	CC	TT	TT	TT
CC	CC	CC	TT	TT	TC
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CC	CC	CC	TC	TT	TT
CC	FL	CC	TT	TC	TT
TC	CC	CC	TT	TT	TT
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CC	CC	CC	TT	CC	TC
CC	CC	CC	TT	CC	TT

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CC	CC	TC	TT	TT	TT
CC	CC	CC	TT	CC	TT
CC	CC	CC	TT	TC	TT
CC	CC	CC	TT	TC	TT
FL	FL	FL	FL	FL	FL
CC	CC	CC	TT	CC	TT
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TC	CC	CC	TT	TC	TT
CC	CC	CC	TT	TT	TT
CC	CC	CC	TT	TC	TC
FL	CC	FL	TT	FL	TC
FL	FL	FL	FL	FL	FL
TC	CC	CC	TT	TT	TT
FL	FL	FL	FL	FL	FL
FL	FL	FL	FL	FL	FL
FL	FL	FL	FL	FL	FL

KEL6	KEL8	DI18	FYP	FY2	GP3A
18	17	17	15	16	16
354	355	355	357	356	356
95.16%	95.43%	95.43%	95.97%	95.70%	95.70%
0	0	0	348	112	263
28	1	2	7	155	89
326	354	353	2	89	4
3.95%	0.14%	0.28%	98.46%	53.23%	86.38%
96.05%	99.86%	99.72%	1.54%	46.77%	13.62%

JK9	Sample FL	Pass Rate
FL	12	0.0%
CC	1	91.7%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	1	91.7%
FL	12	0.0%
TC	1	91.7%
CC	0	100.0%
TT	0	100.0%
TC	1	91.7%
TT	0	100.0%
TC	1	91.7%
TC	0	100.0%
TC	0	100.0%
CC	0	100.0%
TC	0	100.0%
TC	0	100.0%
CC	0	100.0%
TT	0	100.0%
TT	0	100.0%
TC	0	100.0%
TT	0	100.0%
TC	0	100.0%
TC	1	91.7%
TT	0	100.0%
TT	0	100.0%
TC	0	100.0%
TC	1	91.7%
TC	0	100.0%
CC	2	83.3%
CC	0	100.0%
CC	1	91.7%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	1	91.7%
TC	1	91.7%
TC	1	91.7%
TC	0	100.0%
TC	0	100.0%
CC	1	91.7%
TC	0	100.0%
CC	0	100.0%
CC	0	100.0%
TT	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
CC	0	100.0%

TT	0	100.0%
TC	0	100.0%
TC	1	91.7%
TC	0	100.0%
TC	2	83.3%
TT	1	91.7%
CC	0	100.0%
TC	0	100.0%
CC	0	100.0%
TC	0	100.0%
CC	1	91.7%
TC	1	91.7%
FL	12	0.0%
TC	0	100.0%
CC	0	100.0%
TC	0	100.0%
CC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	1	91.7%
CC	1	91.7%
TT	0	100.0%
TT	1	91.7%
TC	0	100.0%
CC	0	100.0%
TT	0	100.0%
FL	12	0.0%
TC	0	100.0%
TT	1	91.7%
TC	0	100.0%
TC	1	91.7%
TC	0	100.0%
TC	0	100.0%
CC	0	100.0%
FL	12	0.0%
TC	0	100.0%
CC	0	100.0%
TT	1	91.7%
TC	0	100.0%
TT	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
TT	0	100.0%
TC	0	100.0%
TC	1	91.7%
CC	0	100.0%
TT	0	100.0%
FL	12	0.0%
TC	1	91.7%
TC	2	83.3%
CC	0	100.0%
CC	0	100.0%
TT	0	100.0%
TC	1	91.7%

TT	0	100.0%
TT	0	100.0%
TC	0	100.0%
TC	0	100.0%
TC	0	100.0%
CC	1	91.7%
TC	1	91.7%
TC	0	100.0%
TC	1	91.7%
TC	0	100.0%
TC	0	100.0%
TC	1	91.7%
CC	0	100.0%
TT	0	100.0%
CC	0	100.0%
CC	1	91.7%
TT	0	100.0%
TC	1	91.7%
CC	0	100.0%
CC	1	91.7%
CC	1	91.7%
TC	0	100.0%
CC	0	100.0%
TC	0	100.0%
CC	0	100.0%
CC	2	83.3%
CC	0	100.0%
TC	0	100.0%
CC	0	100.0%
TC	0	100.0%
TC	1	91.7%
CC	1	91.7%
TC	0	100.0%
TT	1	91.7%
TC	0	100.0%
TC	0	100.0%
CC	0	100.0%
CC	0	100.0%
CC	0	100.0%
CC	1	91.7%
TT	1	91.7%
TT	0	100.0%
TC	0	100.0%
TT	0	100.0%
TT	0	100.0%
CC	0	100.0%
TT	0	100.0%
TT	1	91.7%
TC	0	100.0%
TC	2	83.3%
TC	2	83.3%
CC	0	100.0%
TC	1	91.7%
CC	1	91.7%
TC	1	91.7%
TT	0	100.0%

TC		0	100.0%
TT		0	100.0%
TC		0	100.0%
FL		12	0.0%
TT		0	100.0%
TC		1	91.7%
TC		0	100.0%
TC		1	91.7%
TC		0	100.0%
CC		0	100.0%
CC		1	91.7%
TT		1	91.7%
TC		0	100.0%
TC		1	91.7%
CC		0	100.0%
TT		1	91.7%
CC		0	100.0%
TT		0	100.0%
CC		4	66.7%
CC		0	100.0%
TT		1	91.7%
TC		0	100.0%
TC		0	100.0%
TT		0	100.0%
FL		12	0.0%
TC		0	100.0%
TT		0	100.0%
TT		0	100.0%
CC		0	100.0%
TC		1	91.7%
TC		1	91.7%
FL		12	0.0%
TC		1	91.7%
TC		0	100.0%
TC		1	91.7%
TT		0	100.0%
TT		0	100.0%
TT		0	100.0%
TT		0	100.0%
TC		4	66.7%
TC		1	91.7%
TC		0	100.0%
TC		0	100.0%
CC		0	100.0%
CC		0	100.0%
TC		0	100.0%
CC		0	100.0%
TC		0	100.0%
CC		0	100.0%
TC		0	100.0%
CC		0	100.0%
TT		0	100.0%
TC		1	91.7%
TC		1	91.7%
TT		1	91.7%
TT		0	100.0%
TT		0	100.0%
TT		0	100.0%
TT		1	91.7%

TC		0	100.0%
CC		0	100.0%
TC		0	100.0%
TT		0	100.0%
TT		1	91.7%
TC		0	100.0%
CC		0	100.0%
CC		0	100.0%
TC		0	100.0%
TC		0	100.0%
TC		1	91.7%
CC		1	91.7%
TC		0	100.0%
TT		0	100.0%
TC		0	100.0%
TC		0	100.0%
FL		12	0.0%
CC		1	91.7%
TC		1	91.7%
CC		1	91.7%
TC		1	91.7%
TT		0	100.0%
TC		2	83.3%
TT		0	100.0%
TC		1	91.7%
TT		0	100.0%
TC		0	100.0%
CC		1	91.7%
TC		0	100.0%
TT		2	83.3%
TT		1	91.7%
TC		1	91.7%
CC		0	100.0%
TC		0	100.0%
TT		0	100.0%
CC		0	100.0%
TC		0	100.0%
TC		0	100.0%
CC		0	100.0%
CC		0	100.0%
TT		0	100.0%
CC		1	91.7%
TT		0	100.0%
TC		0	100.0%
TC		0	100.0%
CC		1	91.7%
TT		1	91.7%
TC		0	100.0%
CC		1	91.7%
TC		0	100.0%
TC		0	100.0%
TT		1	91.7%
TT		0	100.0%
TT		1	91.7%
CC		1	91.7%
TC		1	91.7%

		12	0.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		1	91.7%
		0	100.0%
		0	100.0%
		0	100.0%
		12	0.0%
		1	91.7%
		1	91.7%
		1	91.7%
		1	91.7%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		2	83.3%
		1	91.7%
		1	91.7%
		1	91.7%
		0	100.0%
		1	91.7%
		0	100.0%
		1	91.7%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		2	83.3%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		1	91.7%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		0	100.0%
		1	91.7%
		3	75.0%
		0	100.0%
		2	83.3%
		0	100.0%
		0	100.0%

TC		0	100.0%
TT		0	100.0%
TC		0	100.0%
CC		0	100.0%
TC		0	100.0%
FL		12	0.0%
FL		1	91.7%
CC		1	91.7%
TT		0	100.0%
CC		0	100.0%
CC		1	91.7%
TC		0	100.0%
CC		0	100.0%
TT		0	100.0%
FL		12	0.0%
CC		0	100.0%
TC		0	100.0%
TT		0	100.0%
CC		0	100.0%
TC		0	100.0%
CC		0	100.0%
TT		1	91.7%
TT		0	100.0%
TT		0	100.0%
TC		0	100.0%
TT		1	91.7%
TC		0	100.0%
TC		0	100.0%
TC		1	91.7%
TC		0	100.0%
CC		0	100.0%
CC		0	100.0%
TT		0	100.0%
TC		0	100.0%
CC		0	100.0%
TC		0	100.0%
TT		0	100.0%
TC		6	50.0%
FL		12	0.0%
TT		0	100.0%
FL		12	0.0%
FL		12	0.0%
FL		12	0.0%
FL		12	0.0%

JK9
17
355
95.43%
87
178
90
49.58%
50.42%